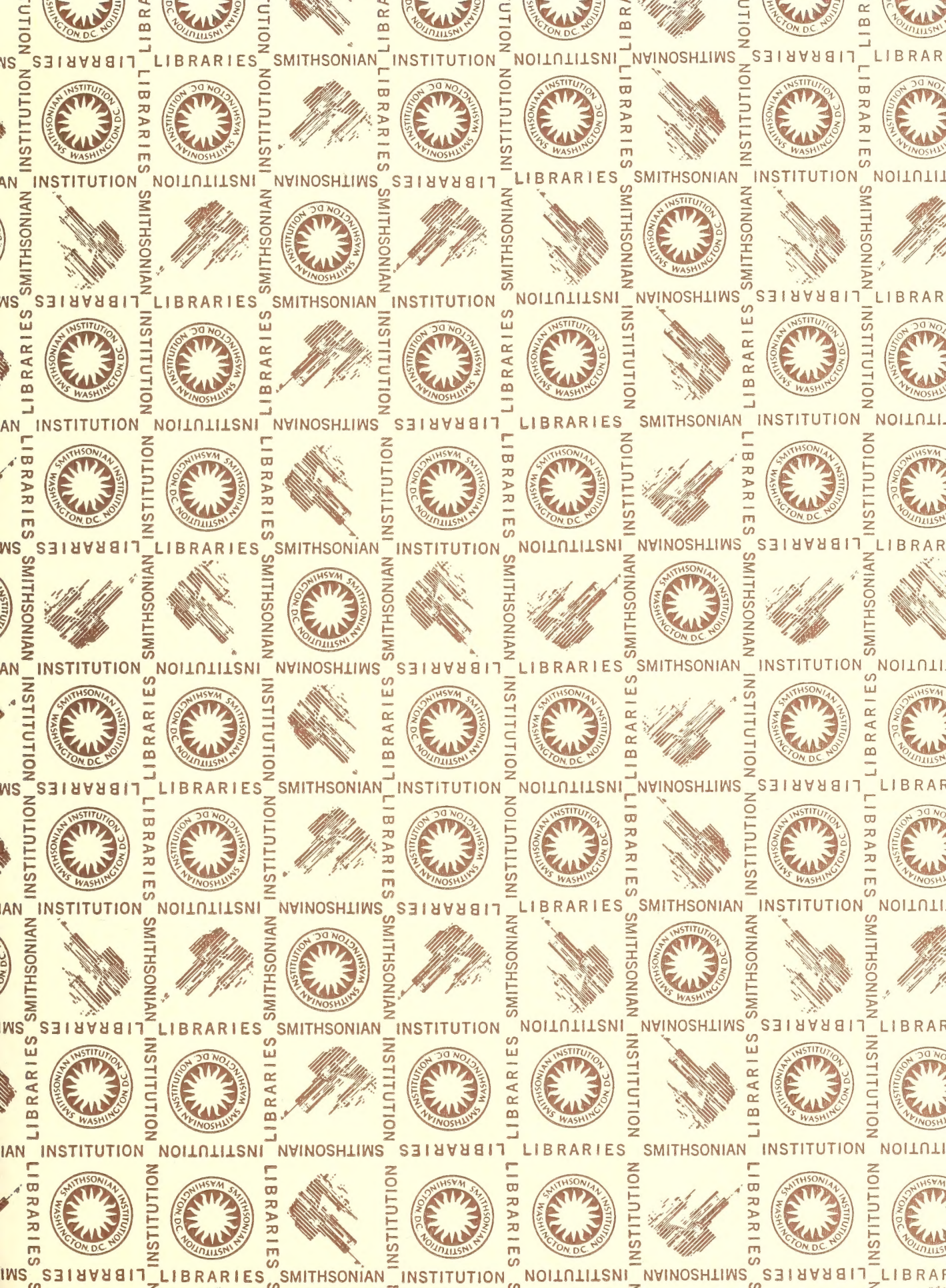








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AMERICAN MALACOLOGICAL BULLETIN

VOLUME 8 NUMBER 1

AUGUST 1990

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Cover. Late veliger of *Atlanta gaudichaudi* Souleyet, 1852. The biology of this species is discussed in a paper by Newman in this issue. This paper is one in a series of papers that appear herein as part of the 1989 American Malacological Union Symposium on the Biology of Pelagic Gastropods.

THE AMERICAN MALACOLOGICAL BULLETIN is the official journal publication of the American Malacological Union.

AMER. MALAC. BULL. 8(1)
August 1990

LIFE CYCLE AND SEXUAL STRATEGY OF *SACCOSTREA CUCULLATA* (BIVALVIA: OSTREIDAE) FROM A HONG KONG MANGROVE

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ABSTRACT

Stems, roots and pneumatophores of Hong Kong mangroves are colonised densely by *Saccostrea cucullata* (Ostreidae). A 13 month study of this oyster has shown that recruitment to the adult population takes place in a single extended phase over mid-summer, correlated with rising sea temperatures and falling salinities. New recruits generally mature into males, creating a heavily male-biased (78%) juvenile sex ratio. Such individuals, maturing in their first year, fertilize a predominantly female biased group of adults (two and three year olds) and then change sex in June to become females themselves. Some two year old oysters change sex, again in June, to become males, but an overall older female biased sex ratio is maintained.

In this habitat, *Saccostrea cucullata* rarely lives beyond three years, such individuals constituting an insignificant component of the total population. Four year old oysters occur only rarely and are covered by successive recruitments.

Such a life history tactic and sexual strategy of alternative hermaphroditism equips this species for opportunistic colonization of the high-zoned mangal and places it firmly, with other species sharing this rigorous habitat, at the *r* end of an *r-K* continuum.

The rock-oyster *Saccostrea cucullata* (Born, 1778) is virtually ubiquitous on Hong Kong shores. It occurs as scattered, isolated, individuals on shores experiencing high exposure to wave action, but forms a dense band in the eulittoral zone of shores experiencing less exposure and is particularly obvious on estuarine shores where it encrusts aerial roots and stems of the seaward fringe mangrove plants *Kandelia candel* (L.) Druce and *Aegiceras corniculatum* (L.) Blanco and pneumatophores of *Avicennia marina* (Forsk.) Vierh. In such a situation it is sympatric with the bysally-attached mytilid *Brachidontes variabilis* (Krauss, 1848) (Morton, 1988).

Saccostrea cucullata is the type species of the genus (Stenzel, 1971) and has a wide Indo-Pacific distribution, from East (Day, 1974; Branch and Grindley, 1979) and South Africa (Lasiak, 1986) to the Pacific Islands, e.g. Guam (Stojkovich, 1977; Braley, 1982), and from New Zealand (Dinamani 1974; 1976) and Australia (Roughley, 1933) to Japan (Torigoe, 1981).

Stenzel (1971) regards *Saccostrea cucullata* as a 'superspecies' and its component 'species', such as *S. glomerata* Gould, 1850, *S. commercialis* Iredale and Roughley, 1933, *S. echinata* Quoy and Gaimard, 1835 and *S. mordax* Gould, 1850, as no more than geographic subspecies, ecomorphs or variants. Morris (1985) adds *Ostrea circumscuta*

Gould, 1850 to this list. Dinamani (1976) could not find one valid morphological character to separate *S. commercialis* (from Australia) from *S. glomerata* (from New Zealand) and further found that many mangrove oysters from the South Pacific could be allied to *S. glomerata* and thus with *S. cucullata*. Similarly, the spiny *S. echinata* from Hong Kong and elsewhere, e.g. Inhaca Island (Macnae and Kalk, 1969), and the similarly spinose *S. kegaki* Torigoe and Inaba, 1981 from Japan (Torigoe and Inaba, 1981) are probably no more than sheltered habitat 'forms' of *S. cucullata* (Morris, 1985). Dinamani (1976) concluded that revision of this oyster assemblage could only be attempted after more detailed studies of all the 'species' of the *S. cucullata* group had been undertaken and laid the foundation for this by investigating the sexual cycle of *S. glomerata* from New Zealand (Dinamani, 1974).

There is a considerable literature on the Caribbean mangrove oyster *Crassostrea rhizophorae* (Morton, 1983), but few detailed studies of its Indo-Pacific counterpart. Such information as is available on reproduction has been reviewed by Lasiak (1986). Hong Kong's rock and mangrove oysters have been identified as *Saccostrea cucullata* (Morris, 1985) and this study is thus of the type species of the genus. Until such time as the taxonomy of all Indo-Pacific 'species' has

been stabilized, only limited comparison with them, e.g. *S. glomerata* (Dinamani, 1974), is possible although the analysis of reproduction in *S. cucullata* from seven locations by Lasiak (1986) has allowed tentative generalizations to be made.

Morton (1985; 1988; 1990a) has studied the life history tactics and sexual strategies of Hong Kong's other mangrove bivalves, i.e. *Polymesoda (Geloia) erosa* (Solander, 1786), *Brachidontes variabilis* and *Gafrarium pectinatum* (L., 1767), and this study permits comparison of *Saccostrea cucullata* with them to determine whether or not they are united by common features that permit colonization and exploitation of the high-zoned mangrove environment.

MATERIALS AND METHODS

Every month (save April 1989) from October 1988 to October 1989, i.e. 13 (minus 1) months, a large sample ($\bar{X} = \sim 500$) of *Saccostrea cucullata* was obtained from the mangrove at Ting Kok, New Territories of Hong Kong. Upon return to the laboratory they were separated into individuals and wet weighed to the nearest 1g. This allowed the construction of monthly total weight frequency histograms. Oysters were subsequently fixed in 10% neutral formalin and, wherever possible, five individuals from each 2g weight class were sectioned. A piece of the body through the gonad was removed from each individual and, following routine histological procedures, sectioned at 6 μm and stained in Ehrlich's haematoxylin and eosin.

Using previously defined criteria of developmental stages (Morton, 1982a, b; 1985; 1988; 1990a; Dudgeon and Morton, 1983), the gonads of *Saccostrea cucullata* were assigned stages of either: 1, primordial; 2, developing; 3, maturing; 4, mature; or 5, spent. Developmental stages for *S. glomerata* (= *S. cucullata*) are illustrated by Dinamani (1974). Data for each sex were also applied to the relative stages of development of hermaphrodites, and used to determine: (a), the timing and size of first maturity; (b), the timing and frequency of sex-reversal; (c), the subsequent pattern of gametogenesis; and (d), the male/female sex ratio, overall, and with increases in size and from month to month. Using a test of proportions, the incidence of males in the total sample, obtained monthly over the 13 month period, was tested against the null hypothesis of equal proportions of both sexes, i.e. both 50%. A Chi-square test was applied to (a), the monthly samples and (b), to the different weight classes to determine if the sex ratio deviated from 1:1 in accordance with these parameters. This has allowed assessment of the reproductive cycle and the sexual strategy adopted.

On each visit, undertaken in mid-morning on a falling tide, three water samples were obtained, following temperature measurement *in situ*. Each sample was held in air-tight, dark glass bottles and upon return to the laboratory analysed as follows: the first to determine dissolved oxygen level; the second, BOD₅; the third salinity, pH, phosphate and nitrate-nitrite. The latter two were determined using a La Motte Water Analysis Kit (La Motte Chemicals Ltd.).

RESULTS

HYDROLOGY

Morton (1990a) reported upon the hydrology of the Ting Kok mangrove in relation to the life cycle of *Gafrarium pectinatum*. The present study adds information to that one. Sea temperature fell to a low of 17°C in January 1989 and then progressively rose to 33.5°C in September 1989 (Fig. 1A). Thereafter it fell. Rainfall was not heavy in 1989, reflected in the low variation in salinity. Salinities were always $> 25\text{‰}$, reaching a maximum of $> 30\text{‰}$ in May and July 1989 (Fig. 1A). Rainfall lowered salinity slightly in late summer and it remained low for October 1989 (28‰). The exaggerated salinity curves typical of Hong Kong's inshore waters (Morton and Wu, 1975) were thus not demonstrated in 1989. pH values were generally low in the winter months of December 1988 - January 1989, i.e. 7.6, and rose to 8.25 in August 1989 (Fig. 1A).

Dissolved oxygen levels were consistently $> 3 \text{ mg}\cdot\text{l}^{-1}$ with high values recorded in spring (March 1989) (5.6 $\text{mg}\cdot\text{l}^{-1}$) and July and August (mid-summer) ($> 5 \text{ mg}\cdot\text{l}^{-1}$) (Fig. 1B). BOD₅ values were all $< 4.0 \text{ mg}\cdot\text{l}^{-1}$ with a low value of 1.2 $\text{mg}\cdot\text{l}^{-1}$ being recorded in January 1989 and a value of 2.4 $\text{mg}\cdot\text{l}^{-1}$ in July 1989. Highest values were recorded from February - June 1989, i.e. $> 3 \text{ mg}\cdot\text{l}^{-1}$ (Fig. 1B).

Nitrate-nitrite levels of 0.88 and 1.14 $\text{mg}\cdot\text{l}^{-1}$ were recorded for the months of October and November 1988, respectively, but thereafter values fell below 0.4 $\text{mg}\cdot\text{l}^{-1}$ and in July, August and September 1989 undetectable levels were present (Fig. 1C). Similarly with phosphate, high levels of 0.45 and 1.05 $\text{mg}\cdot\text{l}^{-1}$ were recorded in October and November 1988,

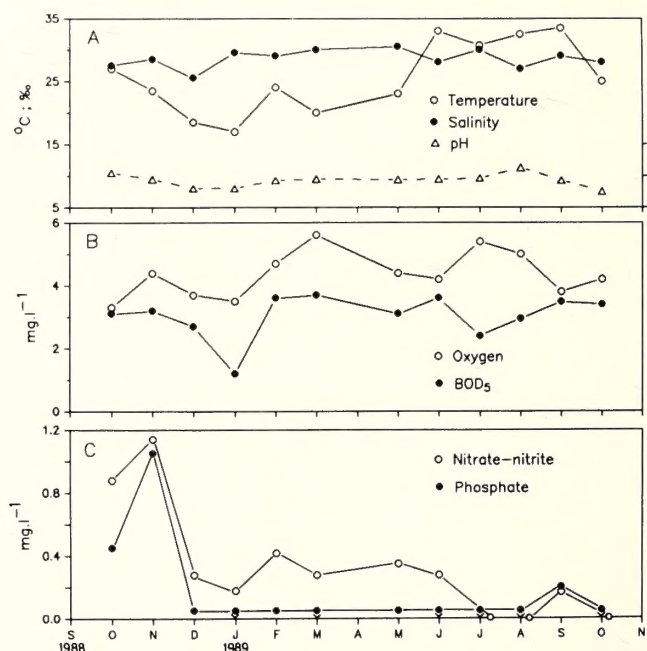


Fig. 1. Water quality parameters at Ting Kok mangrove, Tolo Harbour, Hong Kong, over the period from October 1988 to October 1989. A, temperature, salinity and pH. B, dissolved oxygen and BOD₅. C, Nitrate-nitrite and phosphate.

respectively, but figures generally were $< 0.05 \text{ mg} \cdot \text{l}^{-1}$ thereafter (Fig. 1C).

POPULATION DYNAMICS

The highly variable shell form of *Saccostrea cucullata* precludes use of standard measurements in the construction of normal length frequency histograms that have been the basis for assessment of population dynamics in the Bivalvia. Instead, the total weights of intact animals have been used in the construction of equivalent histograms for this species (Fig. 2). The resultant histograms are thus less discriminating of age cohorts but nevertheless important generalisations can be made. Newly recruited juveniles of $< 1.0\text{g}$ appeared in the population, attached to older individuals, during the period from March - September 1989. This seemed to occur as a single wave of recruitment, undivided into sub-cohorts, and coinciding with spring and summer in Hong Kong. With this information, the histograms were interpreted as follows. In October 1988, the major peak of $\sim 4\text{g}$ represents settlement of that summer's recruits. Other peaks of $\sim 8\text{g}$ and $\sim 13\text{g}$ represent settlements in 1987 and 1986, respectively. The few individuals of 19g and 22g are of unidentifiable age, but clearly older than three years.

From November 1988 to February 1989, a similar population picture was obtained, as also in March 1989 except that the incidence of individuals weighing $< 1\text{g}$ increased to comprise $\sim 12\%$ of the population. These new recruits were easily identifiable for the next six months, with the other age cohorts progressively increasing in weight. Peak recruitment appeared to occur in July and August with older (1988 and 1987) cohorts masked by dramatic settlement and growth of the younger animals. By September 1989 the rate of recruitment had slowed down and by October 1989 a picture similar to that seen in October 1988 was obtained with a large peak of 1989 recruits of $\sim 4\text{g}$ followed by further peaks of $\sim 8\text{g}$ and $\sim 12\text{g}$ representing settlements in 1988 and 1987, respectively, and with a single individual of 17g of indeterminate age. It thus seems that *Saccostrea cucullata* lives for ~ 2 years, with only a few identifiable animals (1 - 2%) entering their third year and with even fewer ($< 1\%$) entering their fourth and possibly fifth years.

GROWTH

Analysis of the histograms presented in figure 2 and identification of cohort peaks for each of the recruitment years has allowed an assessment of growth (Fig. 3). Somatic growth appears restricted to spring and summer months from March to September with no overall increasing trend being evident for all cohorts from October - February. With settlement occurring over the same period, it is clear that the newly recruited oysters reach an approximate weight of 5g very quickly. In their second year they attain a weight of $\sim 12\text{g}$ and in their third a weight of $\sim 15\text{g}$. Somatic growth and gonad maturation, over the time frame monitored, thus coincide with rising sea (and air) temperatures, reduced salinities and high oxygen levels.

SEXUAL CYCLE

Analysis of sectioned gonads of *Saccostrea cucullata* shows that the species completes one sexual cycle each year (Table 1). In October 1988, the majority of gonads were either mature (Stage 4) or spent (Stage 5). This situation continued until November but by December 1988 the gonads were largely primordial (Stage 1) or immature (Stage 2). By February - March, gonads were maturing (Stage 3) with a majority reaching sexual maturity (Stage 4) in April - May 1989. Gonads were mature from this time until approximately August - September 1989, with the incidence of spent individuals increasing in October 1989, as in October 1988. For *S. cucullata*, therefore, spring in Hong Kong is the period of sexual maturation, summer when the gonads are mature and autumn and winter the time of gonadal decline and gametogenesis initiation respectively.

SEXUAL STRATEGY

Table 1 shows the stage of sexual maturity of sectioned individuals of, wherever possible, five individuals belonging to each 2g weight class. The overall sex ratio is biased towards males (50.5% males vs 36.6% females). This is significantly different ($P = < 0.001$). There is, moreover, a significant difference ($P = < 0.001$) between the period from October 1988 to May 1989 and that from June 1989 to October 1989 with males predominating in the former and females in the latter.

Significantly also ($P = < 0.001$), the sex ratio changes with increasing size and thus age. In the weight class from 0 - 1.9g, 77.6% of all individuals were male. This figure progressively decreased while the corresponding figures for females increased to 77.8% in the 16.0 - 17.9g weight class. In June 1989, however, 27.1% of all individuals were hermaphroditic. Such hermaphrodites could be divided into two sub-groups in the weight ranges 2.0 - 7.9g and 10.0 - 15.9g. The former hermaphrodites were spent males (Stage 5) and either maturing (Stage 3) or mature females (Stage 4). The latter were spent females (Stage 5) and either developing (Stages 1-3) or mature males (Stage 4). Such hermaphrodites constituted an average of 2.8% of the 2.0 - 7.9g individuals, and 5.8% of the 10.0 - 15.9g individuals, i.e. the latter were relatively twice as numerous as the former in their respective weight classes.

From the foregoing, a picture of the sexual strategy can be constructed. The majority (78%) of newly recruited individuals mature into males during their first year of life leading to a juvenile male biased sex ratio from October to May. In June, many juveniles, now one year old, change sex into females leading to a female biased sex ratio from July to October. Oysters in their second year of life, i.e. $> 8.0 - 9.9\text{g}$, are thus predominately females. In June of the following year, some spent females change sex again into males. Most do not, however, because older weight classes still are firmly female sex-biased.

LIFE HISTORY TACTICS

From the evidence before us of changes in the population structure and sexual strategy of *Saccostrea cucullata* at

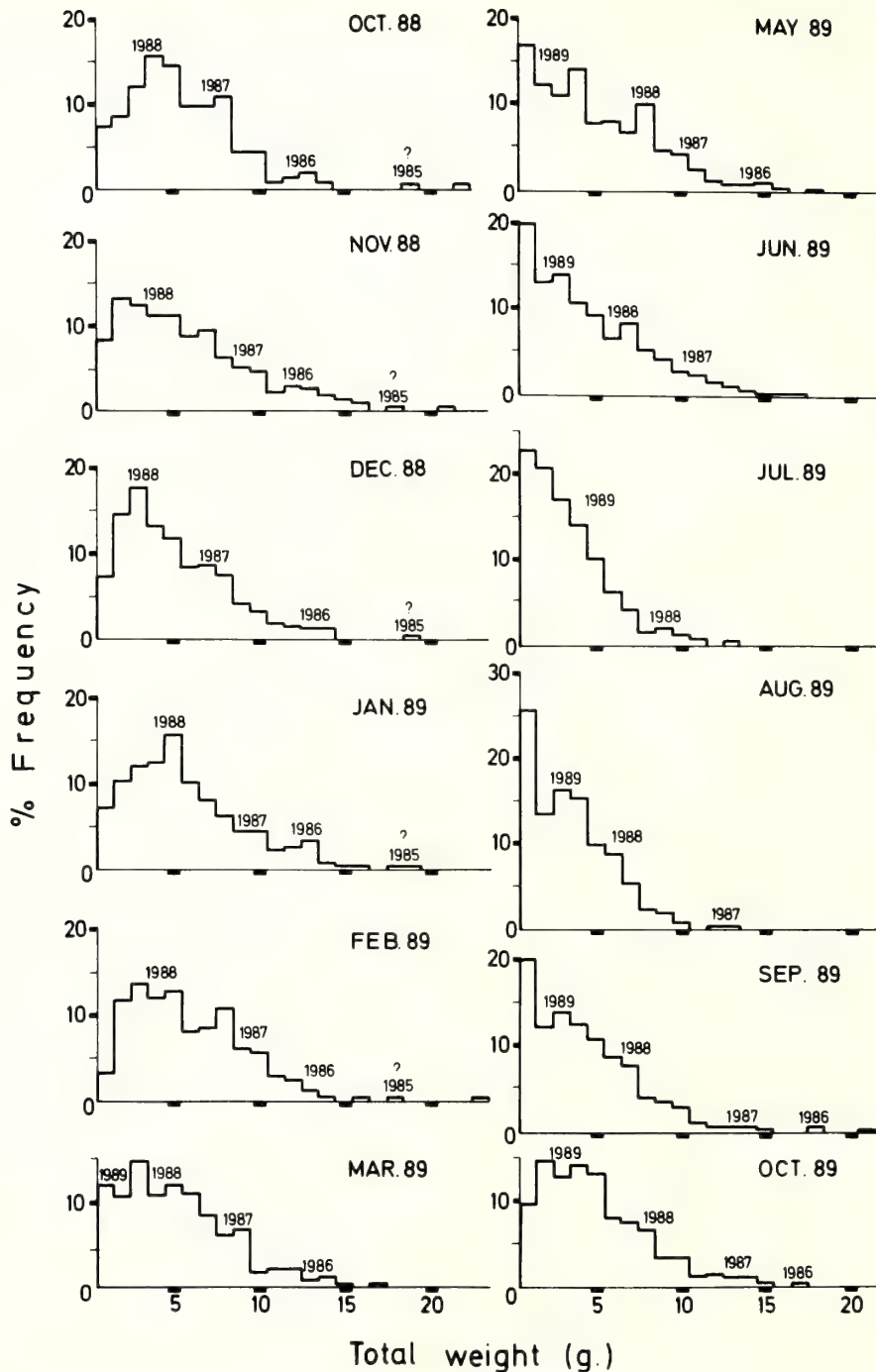


Fig. 2. The population structure of *Saccostrea cucullata* at Ting Kok mangrove, Tolo Harbour, Hong Kong over the period from October 1988 to October 1989. Dates above peaks indicate assessed years of recruitment.

Ting Kok, and with associated hydrological data, a picture of the life history can be proposed. *S. cucullata* juveniles are recruited into the adult population in mid-summer when sea water temperatures are consistently $> 30^{\circ}\text{C}$ and salinities could be lowered due to summer rains. It is unknown if spawning and recruitment occur as a distinct series of events related

to the lunar cycle as is seen in other oysters (Walne, 1974; Andrews, 1979). They grow rapidly to a weight of about 5g and then growth ceases over the autumn period. Gametogenesis begins in December, primordia developing some six months after settlement. Most juveniles mature into males (77.6%).

In late winter-early spring, when temperatures are rising from their low of $\sim 17^{\circ}\text{C}$ in January and salinities are generally high, juveniles begin to grow again, their gonads maturing simultaneously, and mature and spawn in early to mid-summer at a weight of $\sim 7.5\text{g}$. Many of the spent males develop female gonads in June, so that a high percentage of hermaphrodites is recorded (27.1%) and a majority of the population, by a weight of 8.0 - 10.0g, is female (55.2%). It is perhaps significant that sea temperatures rose dramatically from 23°C to 33°C in May and salinities fell slightly over the same period from 30.5‰ to 28‰. The second year female oysters, now covered by a mass of settling juvenile males, overwinter, their gametes developing slowly. Somatic growth begins again in December-January and the females are mature by May. At this time, they spawn with the eggs fertilized externally by the now similarly mature males. In June of the following year, at a weight of $\sim 12\text{g}$, some of the spent females change sex into males so that in this month individuals with a weight between 10.0 - 16.0g are maturing male and spent female hermaphrodites. Such individuals then enter their third year of life, overwintering with little growth and beginning to mature, either into males or females, in early spring. Shell growth begins again, later, in March to a modal weight of about 15g. Only occasionally were such individuals collected. For most individuals in the population, therefore, the life span is two years in which they are first predominately male and then predominately female with the sex change occurring in June of each year.

DISCUSSION

The early research of W. R. Coe established the framework for our understanding of sexual strategies employed by the Bivalvia, notably the oysters (Coe, 1943).

Coe's review of oysters of the genus *Crassostrea*, i.e. *C. virginica* and *C. gigas*, demonstrated alternative sexuality with annual changes in sex. Amemiya (1929) showed for *C. gigas* that 25% of the females and 60% of the males in his experiment reversed their sex during one winter. Andrews (1979) has shown that about 96% of first year individuals (35 mm) of *C. virginica* are males with the reverse % in older oysters. Galtsoff (1964) estimates that about 10% of mature individuals of this species change sex annually.

Roughley (1933) concluded that the Australian oyster, *Ostrea commercialis* (= *Saccostrea cucullata*) spawned first as males, though there appeared to be 2.7 times as many females as males in the population. Dinamani (1974) investigated the rock oyster *Crassostrea glomerata* (= *S. cucullata*) (Stenzel, 1971; Dinamani, 1976) and showed a similar situation with reproduction occurring over a single period in summer (January and February in southern hemisphere New Zealand) and with the majority of oysters of age one year and below male, and a greater percentage older than one year, female. Dinamani (1974) also gives data on gametogenesis of this oyster and provides photomicrographs of the gonads at various stages of development. Literature on reproduction in the oysters has been reviewed by Andrews (1979). The situation seen in *S. cucullata* from Hong Kong conforms to the pattern established for *C. virginica* and other oviparous oysters of this genus, although the number of spawning periods evident in any one year varies with species and location. *C. madrasensis*, for example, spawns twice in spring and autumn, in any one year in India (Stephen, 1980).

Most studies of oysters have used cultivated individuals or those growing upon rocks; little information is available for Indo-Pacific species in the mangrove component of their habitat continuum. Available information has been reviewed by Morton (1983). The western Atlantic mangrove oyster

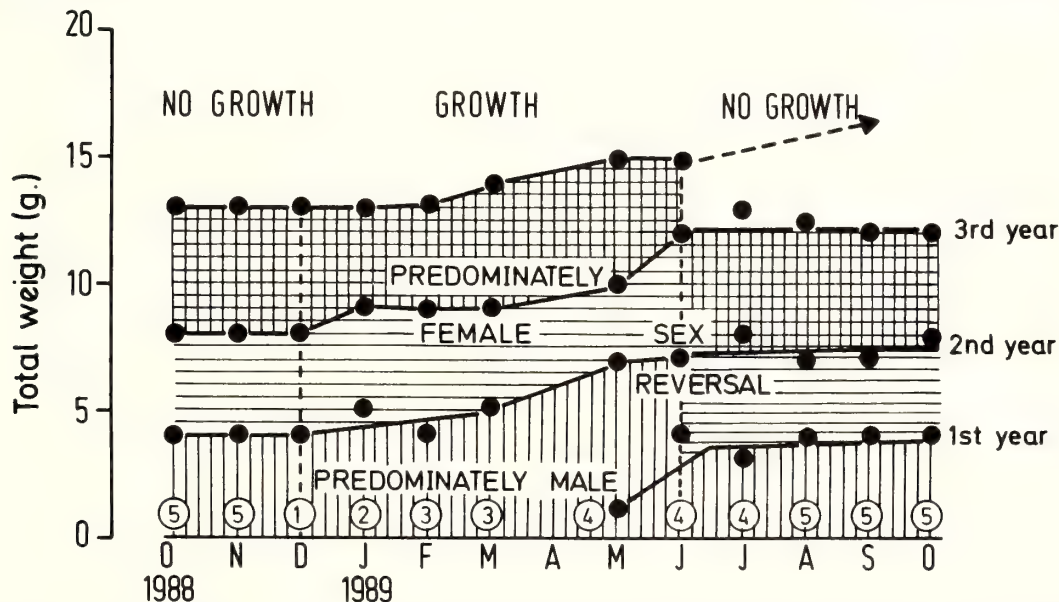


Fig. 3. The pattern of growth of *Saccostrea cucullata* at Ting Kok mangrove, Tolo Harbour, Hong Kong, as determined from the monthly weight frequency histograms (Fig. 2). Superimposed upon these data is the predominant sex of each year class and the monthly stage of sexual maturity (1-5), as obtained from Table 1.

Table 1. Percentage incidence and stage of gonadal development of immature individuals (●) of *Saccostrea cucullata*, males (○), females (□) and hermaphrodites (◇) and mean gonad condition (1-5) of individuals ranging in total weight from 1.0-1.9 g - 16.0-17.9 g obtained at monthly intervals from October 1988 to October 1989 from the Ting Kok mangrove, Tolo Harbour, Hong Kong. Figures at the bottom of each column represent the percentage incidence of immature individuals, males, females and hermaphrodites in that size category.

Month	Total weight (g)									% Incidence				Predominant Sexual stage		
	0 - 1.9	2 - 3.9	4 - 5.9	6 - 7.9	8 - 9.9	10 - 11.9	12 - 13.9	14 - 15.9	16 - 17.9	●	♂	♀	♀	♂	♀	♀
Oct. 1988	5 ● 1 4/5 4/5	4/5 4 5 5 1	5 5 4/5 5	1 5 5 5 5	5 5 5 5 5	5	1 1			3.7	59.2	37.0	-	5	5.1	-
Nov.	4/5 1 5 4/5 4/5	4/5 4/5 4/5 4/5 4/5	5 1 5 5 5	5 1 5 4/5 4/5	2 5 5 5 5	1 5 5 4 5 5	2 5 5 ●	5 5 5 5	2	2.6	65.8	31.6	-	5	5.1	-
Dec.	5 3 1 5 ●	● 4/5 1 3 4	1 1 1 3/4 1	1 1 1/2 1 1	● 1 3 ● ●	1/2 1 1 3/4 1	1 2 2 1/2 1/2			14.3	77.1	8.6	-	1	1	-
Jan. 1989	1 2/3 2 4 1	3 ● 4 1 2	● 4 ● ● 2	2 1 2 1 ●	1 2/3 2 1 1	● ● ● ● ● ●	2 1/2 3 1/2 ●		1	30.6	50.0	19.4	-	2	1-2	-
Feb.	● ● ● ● 3	3 3 4	3 3/4 ● 3/4 3/4	● 3/4 3/4 3 ●	1 2/3 2 2 1	1 1 2 2 2	● ●	1	1	28.1	62.5	9.4	-	3	2	-
Mar.	3/4 3/4 1 3 ●	● 1 1 3 3/4	1 3/4 3 4 3	● 2 3 2 3	2/3 4 2 2 4	● 4 3 4 3/4	2 2	3	3/4	11.8	50.0	38.2	-	3-4	2-3	-
May	3/4 3 3 3 ●	4 4 3 3/4 4	3 3 4 1 4	3 2 2 4 2	3 4 4 4 ●	4 2 4 4 4 4	3 4 4 4	3 4 4 4 4	4	4.9	53.6	41.5	-	4	3-4	-
Jun.	5 5 3 3 3/4	5 4 4 4/5 4/5	4 5 4 4/5 4/5	4 5 5 4/5	4 5 4/5 4/5	4 4 4 4/5 4/5	4 4 4 4/5 4/5	4 4 4 4/5 4/5	4	-	32.4	40.5	27.1	4-5	4	5/4 1-4/5 = 3/5
Jul.	3/4 3/4 3/4 3/4 3/4	3/4 4 4 3/4 4/5	4 4 3 5 ●	3/4 2 3/4 3/4 4	3/4 1 3/4 3 5	2 4 4 3	1 4			3.3	36.7	56.7	3.3	4	4	5/1
Aug.	4 4 5 ●	● 1 1 5 4	5 5 4/5 4 ●	4 3 ● 4 2/3	4 2 4/5 5	1 ●				20.0	36.0	44.0	-	4-5	4-5	-
Sep.	5 5 5 5	4/5 4/5 4/5 5 ●	5 4 4 5 4	5 5 5 4/5	4 4 4 5	4 4 4 4	4 4/5 4/5	4/5	4/5	3.2	38.7	58.1	-	4-5	4-5	-
Oct.	4 4 4/5 5 5	5 4 4 5	4 4 5 4 4	5 5 5 5 5	5 5 5 5 1	5 4 5 1 1	4 5 5 1 1	5 5	4 5	1.4	43.6	53.8	-	4-5	4-5	-
Incidence %										15.5	10.3	10.2	8.6	6.9	14.0	10.2
♂										77.6	62.1	61.0	48.3	37.9	34.0	33.3
♀										6.9	24.1	25.4	41.4	55.2	46.0	51.3
♀										-	3.4	3.4	1.7	-	6.0	5.1
										-	-	-	-	-	6.2	-
										10.3	50.5	36.6	2.5	x̄		

Key: ● = Immature individuals
○ = Males
□ = Females
◇ = Hermaphrodites
1-5 = Stage of sexual development

Crassostrea rhizophorae was studied by Nascimento *et al.* (1980) in Brazil who showed that this species was also predominately male in its first year, with sex reversal occurring later. In its second year, *C. rhizophorae* achieves a shell length of 40mm, which is of marketable size. *C. rhizophorae*, however, breeds over the winter months from late summer (August - October) to March at a time of rising salinities (Bacon, 1970; Rojas, 1972; Wedler *et al.*, 1978).

The West African oyster, *Crassostrea gasar* (Adanson), in Sierra Leone, reproduces in winter (Hunter, 1969). In south west India, *C. madrasensis* (Preston) has two peaks of spawning; a major one occurring from April - June with a second minor peak in September - October. This sub-tidal species

is separated in the Indian Sub-continent from the intertidal *Saccostrea cucullata* (Ansari and Ahmed, 1972).

It is possible that in the Indo-Pacific there is a 'super-species' of intertidal oyster generally referred to as *Saccostrea cucullata* (Stenzel, 1971) but with a wide variety of geographic names attached to it. Lasiak (1986) has reviewed the available information on "*S. cucullata*" from seven locations throughout its broad range and shown that reproductive activity is continuous in tropical populations in Singapore (Ling, 1970), India (Awati and Rai, 1931; Nagabhushanam and Birdarkar, 1977), Pakistan (Asif, 1980) and Guam (Braley, 1982). Sub-tropical or more temperate populations as for example in Australia (Roughley, 1933) and Southern Africa (Lasiak, 1986), however,

have a single annual cycle with spawning taking place in early summer. In Hong Kong, *S. cucullata* conforms to this latter pattern. Lasiak (1986) could detect only a few hermaphrodites (3 out of 333 individuals sectioned) in the population of *S. cucullata* from South Africa. In Hong Kong, the species is oviparous and exhibits alternative sexuality, a percentage of the population changing sex each year.

Spawning and recruitment in the Bivalvia are sequential but both are influenced by a variety of hydrological parameters. In the absence of confirmatory studies that all these oyster 'species' are synonymous and can be categorized under the name *Saccostrea cucullata*, caution must be exercised in comparing the results obtained in Hong Kong with those obtained from elsewhere. However, a few generalizations can be made. The gonadal cycle of *S. cucullata* appears locally to be linked to temperature changes, as with this species in South Africa (Lasiak, 1986) but with this clearly not the case in tropical populations, e.g. Singapore (Ling, 1970). In tropical populations too, spawning can be induced by salinity reductions associated with heavy monsoon rains (Ling, 1970; Nagabhushanam and Birdarkar, 1977; Stephen and Shetty, 1981) but this is not the case either in South Africa (Lasiak, 1986) or in Hong Kong where local changes in salinity in the mangrove habitat (at least in the, admittedly dry, year of 1989) following rainfall are not so dramatic.

Saccostrea cucullata occurs locally in a variety of subhabitats and clearly dominates the seaward mangrove habitat, encrusting stems, roots and pneumatophores. Of local ecological interest could be an answer to the question: how is this achieved? What aspects of the life history strategy of this species equip it for habitat dominance? In this context, Morton (1987, 1990b) has reviewed the life history tactics of southern Chinese fresh and brackish water bivalves and the sexual strategies of a wide spectrum of local bivalves.

Saccostrea cucullata locally fits into the *r* end of an *r-K* continuum (MacArthur and Wilson, 1967; Pianka, 1970), being highly opportunistic. The species grows fast and achieves sexual maturity in its first year of life. Growth of newly recruited juveniles halts over winter, but with the approach of spring at a time of high salinities and rising temperatures, somatic growth and gonad maturation proceed concurrently. This could be facilitated by the high levels of nutrients in the mangrove habitat (Fig. 1C). Juveniles predominantly mature into males as Morton (1988, 1990a, b) has shown for two other, albeit dioecious, seaward mangrove bivalves, i.e. *Brachidontes variabilis* and *Gafrarium pectinatum*. These species, too, have a male-biased juvenile sex ratio, which, it is thought, progressively changes through enhanced juvenile mortality into a female dominance in subsequent year classes. Like *S. cucullata*, these species live for two to three years with the majority of all individuals in all species rarely living beyond two years. The population histograms for *S. cucullata* suggest that the species can live for greater than three years, but only a few achieve this, successive recruitments possibly suffocating older individuals as new recruits settle upon them.

Thus in terms of the Bivalvia, local seaward mangroves are colonized by a guild of *r*-strategists, *Saccostrea cucullata*

and *Brachidontes variabilis* being sympatric, the latter epibyssate on the former. Such a situation is different from the landward mangrove species, *Polymesoda (Geloina) erosa*, which occupies the *K* end of the *r-K* continuum, being long-lived, dioecious and with an overall female biased sex ratio (Morton, 1985, 1990b).

In subtropical Hong Kong, all four mangrove associates breed in summer, *Polymesoda* and *Saccostrea* in a single phase in mid-summer, *Brachidontes* and *Gafrarium* in two phases in early summer and late summer/autumn. All species are tropical/subtropical, but it is thought possible that reproduction in *Brachidontes* and *Gafrarium* is divided into two phases by low summer salinities inhibiting either gametogenesis or spawning or both (Morton, 1987).

In this context, Morton and Chan (1989) have shown that *Polymesoda erosa* is oligohaline and *Gafrarium pectinatum*, marine stenohaline, the latter, but not the former, therefore clearly responding dramatically to salinity fluctuations. *Saccostrea cucullata* and *Brachidontes variabilis* are, however, marine euryhaline and thus osmoconform to a wide range of salinities.

A combination of morphological, behavioural, physiological and reproductive adaptations thus allow such bivalves to exploit the high-zoned mangrove habitat, throughout much of the Indo-Pacific. Such adaptations are reflected in life history tactics with the numerically dominant species, *Saccostrea cucullata*, opportunistically colonizing the lower components of the mangrove habitat.

ACKNOWLEDGMENTS

I am grateful to Mr. M. H. Lam and Ms. Lisa Ng for assistance with analysis of the samples and Mr. S. F. Leung for histological assistance.

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Date of manuscript acceptance: 9 March 1990.

ECOLOGICAL ANALYSIS OF THE LIVING MOLLUSCS OF THE TEXAS PANHANDLE

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ABSTRACT

The living molluscan fauna of the Texas Panhandle includes a total of 43 species: nine freshwater gastropods, 24 terrestrial gastropods (including one slug), and ten freshwater bivalves. Except for one terrestrial gastropod and one bivalve, all of these species are native to the central United States. Five freshwater and seven terrestrial gastropods, and six freshwater bivalves are here reported living in the Texas Panhandle for the first time. One terrestrial gastropod is a new record for Texas. Occurrence of freshwater gastropods and pelecypods is limited by quality and quantity of surface waters, whereas terrestrial gastropods are limited by distribution of surface soil moisture and cover objects. Highest diversity terrestrial faunas are associated with woodlands of plains cottonwood, *Populus sargentii*, but the mesic-adapted species can be found in moist floodplains even in the absence of trees.

Until recently, the living molluscan fauna of the Texas Panhandle has been poorly known. General biological interest in this area has been limited in the past, at least partially because of a presumed low species diversity. The Texas Panhandle presents an extreme environment for both terrestrial and freshwater molluscs. The general aridity of the climate is made even more rigorous for molluscs by wide seasonal thermal extremes. For this study, the Texas Panhandle was defined as that part of Texas between the 100th and 103rd meridians and north of 34°18'40"N, corresponding to a series of county boundaries between Cottle/Childress on the eastern end and Bailey/Parmer on the western end (Fig. 1).

STUDY AREA

Two major physiographic regions exist in the Texas Panhandle. The greater part of the area is within the High Plains where Quaternary eolian deposits overlie the Ogallala Formation of the Miocene and Pliocene. The High Plains is a nearly level plain rising gradually to the west. Potential natural vegetation for most of the Texas Panhandle is short and mixed grass prairies as observed by early European explorers (Strout, 1971; Flores, 1984). Woody plants were few in species number and were generally restricted to canyons and steep slopes (Palmer, 1920).

The eastern portion of the Panhandle is the second physiographic region and contains a mosaic of two units: 1) Permian redbeds or "badlands" with limited soil development and a mixed grassland/scrub community; 2) Quaternary

alluvial plains with deep soils that naturally supported mixed grass prairie communities. The Permian badlands extend westward along the Canadian River. Gallery woodlands exist on Quaternary alluvial deposits along the larger water courses. Portions of the northeastern and southwestern Panhandle contain sand hills and ridges overlying the Ogallala and support a mixed grass/herbaceous prairie community or a stunted woodland community.

Analysis of the herpetofauna and woody flora reveals a mixed biota with regard to biogeographical affinities of the constituent species. Species present include those characteristic of central prairies, southern savannahs and brushlands, and western montane areas along with a few representatives of eastern forests (Palmer, 1920; Fouquette and Lindsay, 1955).

The Texas Panhandle has a warm temperate, semiarid, continental climate. Mean annual temperature at Amarillo is 13.9°C, with record extremes ranging from -26.7°C to 41.1°C. The growing season averages 190 days from 17 April to 24 October. Summer days produce high temperatures (64 days with maximum above 32.2°C), but radiational cooling results in cool nights and early morning hours. Winter weather is characterized by strong cold fronts with occasional blizzards, but these fronts are usually followed by a warming trend after several days. Freezing temperatures occur on 110 days annually. Average annual precipitation is 485 mm with a record of 1110 mm in 1923. Most precipitation occurs during thunderstorms, which occur on an average of 49 days annually. The above climatic data are taken from the Natural Fibers

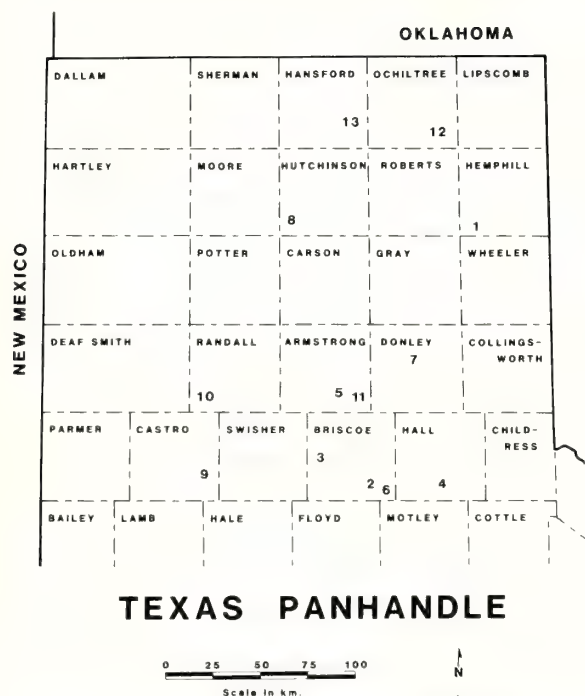


Fig. 1. Panhandle of Texas giving collection localities referred to in text.

Information Center (1987).

The Texas Panhandle is drained by the Red/Canadian drainages of the Mississippi River system except for the extreme southwestern corner which is drained by Running Water Draw of the Brazos River drainage. Flowing waters in this region are quite restricted in width, depth, and permanence. Large streams have low flows with high concentrations of suspended red silt/clay and dissolved salts. Such water conditions are natural (E. James *In*: Thwaites, 1905), but modern land use practices have further decreased the water quality of these streams. Water flows in these larger streams have been described as being "either dry or raging current" (Gould, 1906:42). Small feeder creeks are generally intermittent but a few creeks have perennial flows supplied by "sweetwater" springs supplied by the Ogallala Aquifer. Natural lakes are limited to the numerous playas on the High Plains surface (Guthery and Bryant, 1982).

LITERATURE REVIEW

Neck (1984) published the results of a survey of living terrestrial gastropods of various canyons of the Eastern Caprock Escarpment. Most published records (Henderson, 1909; Strecker, 1910; Walker, 1915; Clarke, 1938) were rejected as being samples of flood debris containing shells of undeterminable, probably fossil, origin. Only a few published records of gastropods from this area were accepted as representing modern living populations (Pratt, 1965; Metcalf *In*: Franzen, 1971; Metcalf *In*: Bequaert and Miller, 1973). The opinions of Neck (1984) and the records accepted therein produced a list of 12 species of terrestrial gastropods known to be living along the Eastern Caprock Escarpment; two additional species were

known from other areas of the Texas Panhandle.

A subsequent review of terrestrial gastropods of the eastern United States (Hubricht, 1985) included several new records from the Texas Panhandle. The reports of *Vallonia pulchella* (Müller), *V. cyclophorella* (Sterki), and *V. perspectiva* Sterki from Potter and Randall counties (Hubricht, 1985:63, 65, 67) probably represent reworked fossil shells or introduced populations (Pilsbry, 1948; Bequaert and Miller, 1973; Pierce, 1975; Neck, 1989a) and are not accepted as native members of the living Panhandle fauna. Only limited records of urban molluscs of the Texas Panhandle are available (Neck, 1989a). Hubricht (1985) reported *Succinea forsheyi* I. Lea and *S. indiana* Pilsbry from Hemphill and Sherman counties, respectively, although taxonomic uncertainty prevents complete understanding of species relationships of the succineid snails. The Sherman County record was originally referred to *S. vaginacontorta* Lee by Franzen (1971), a taxon synonymized with *S. indiana* by Hubricht (1985).

Records of freshwater gastropods from this area are also rare (Fullington, 1978), although *Physella* sp. and *Planorbella* sp. were observed in playas as early as 1876 (E. H. Ruffner *In*: Baker, 1985). Clarke (1938) reported *Physella virgata* (Gould) living in "Paladura Creek [northeast] of Canyon," which is actually the Palo Duro Canyon of the Prairie Dog Town Fork of the Red River. Singley (1893) reported *P. virgata* and *Planorbella trivolis* (Say) from a playa. Strecker (1910) reported *P. virgata* and *P. tenuis* (Dunker) from creeks and tanks in Armstrong County. A survey of playa lakes of the Llano Estacado of Texas and New Mexico (Sublette and Sublette, 1967) revealed the presence of *P. trivolis* in five of 12 playas surveyed in the current study area. Rhodes and Garcia (1981) reported "snails (mostly *Physa* sp. and *Lymnaea* sp.)" from all eight playas that they surveyed from Castro and Swisher counties. Neck (1982) reported *P. virgata* and *P. trivolis* from Lake Theo, Briscoe County. Several freshwater gastropods have been reported from playas in Castro County (Neck and Schramm, in press).

Freshwater bivalves are less widely distributed in the Texas Panhandle than are freshwater gastropods. Clarke (1938) reported living *Sphaerium striatinum* (Lamarck) [as *S. sulcatum* (Lamarck)] and *Uniomus tetralasmus* (Say) from the Prairie Dog Town Fork of the Red River, Randall County. The occurrence of *U. tetralasmus* in a stream of variable volume is related to the ability of this species to withstand complete desiccation of its habitat. Neck (1982) reported *S. striatinum* and an introduced population of *Anodonta grandis* Say in Lake Theo, an artificial impoundment on Holmes Creek, Caprock Canyons State Park, Briscoe County. Populations of the widespread exotic Asian clam, *Corbicula fluminea* (Müller), occur in Greenbelt Reservoir, Donley County, and Lake Meredith, Hutchinson County (Neck, 1987b). A single record of *S. striatinum* from a playa is known (Neck and Schramm, in press).

METHODS AND RESULTS

Soil or benthic samples were either obtained by the author or received from other field workers as noted. Mollusc

Table 1. Occurrence of living nonmarine molluscs in the Texas Panhandle (includes both published reports and reports herein): O = previously published reports of living terrestrial accepted by Neck (1984); A = records in Singley (1893); B = Strecker (1910); C = Clarke (1938); S = Sublette and Sublette (1967); F = Fullington (1969, 1978); N2 = Neck (1982); N4 = Neck (1984); H = Hubricht (1985); N7 = Neck (1987b); NS = Neck and Schramm, in press; U = urban snails in Neck (1989a); single numbers refer to locality numbers in text; taxa are presented in phylogenetic sequence within each group.

Species	O	A	B	C	S	F	N2	N4	H	N7	NS	U	1	2	3	4	5	6	7	8	9	10	11	12	13
FRESHWATER GASTROPODS																									
<i>Hebetancylus excentricus</i>													X												
<i>Fossaria cockerelli</i>											X						X								
<i>F. dalli</i>																								X	
<i>F. modicella</i>																			X						
<i>F. parva</i>													X												
<i>Physella virgata</i>		X	X	X		X	X		X	X		X				X		X	X	X	X	X	X	X	X
<i>Gyraulus parvus</i>						X				X								X							
<i>Planorbella tenuis</i>			X			X			X				X	X			X		X		X				X
<i>P. trivolvis</i>		X			X		X				X											X			
TERRESTRIAL GASTROPODS																									
<i>Vallonia gracilicosta</i>	X							X							X							X			X
<i>V. parvula</i>	X							X	X	X			X						X			X	X	X	X
<i>Pupoides albilabris</i>								X	X					X	X				X			X	X	X	X
<i>Gastrocopta cristata</i>								X	X			X	X	X	X						X	X			X
<i>G. pellucida</i>								X	X				X	X					X			X	X	X	X
<i>G. procera</i>								X					X	X					X			X	X	X	X
<i>G. armifera</i>								X	X				X						X			X	X	X	X
<i>G. abbreviata</i>									X						X				X						X
<i>G. tappaniana</i>													X						X						
<i>Vertigo ovata</i>														X					X						
<i>Succinea forsheyi</i>									X																X
<i>S. indiana</i>	X								X										X			X			
<i>Catinella cf. avara</i>								X					X						X		X				X
<i>Oxyloma retusa</i>																		X							
<i>Helicodiscus parallelus</i>								X														X			X
<i>H. singleyanus</i>																						X	X		X
<i>H. inermis</i>								X	X				X	X	X				X						
<i>Deroceas laeve</i>								X							X				X		X			X	X
<i>Hawaii minuscula</i>															X									X	
<i>Zonitoides arboreus</i>																						X			
<i>Euconulus trochulus</i>													X		X				X						X
<i>Rumina decollata</i>												X													
<i>Rabdotus dealbatus</i>	X							X	X													X			
<i>Stenotrema leai</i>	X								X																
BIVALVES																									
<i>Anodonta grandis</i>							X											X		X					X
<i>A. imbecilis</i>																				X					
<i>Unio merus tetralasmus</i>				X																		X			
<i>Sphaerium striatinum</i>				X			X				X						X								
<i>Musculium transversum</i>																									X
<i>Pisidium casertanum</i>																			X						X
<i>P. compressum</i>																			X						
<i>P. nitidum</i>																									X
<i>P. punctiferum</i>																									X
<i>Corbicula fluminea</i>										X											X				

shells were extracted by hand picking and screening with soil sieves (nested series of #4, #8, #16, and #30) and identified. Below is a description and location of each collecting site with a statement of the significance of recovered species. Species recovered in the current study as well as those from previously published reports are tabulated in Table 1. Species for which living representatives are recorded for

the first time from the Texas Panhandle are discussed below (see appendix 1).

LOCALITIES

Locality 1. Modern alluvium of Washita River at U.S. 83, 21.4 km south of Canadian, Hemphill County; area with grasses, shrubs at 770 m elevation; sampled 5 Apr 1984. Soil samples

were taken from underneath downed wood. *Fossaria parva* (Lea), *Hebetancylus excentricus* (Morelet), *Gastrocopta tappaniana* (C. B. Adams), and *Euconulus trochulus* (Reinhardt) from this site were reported living in the study area for the first time. Also significant in this collection was the occurrence of *Gastrocopta armifera* (Say) in an herbaceous community; previously, this species had been found in the Texas Panhandle only in habitats dominated by trees with abundant leaf litter on soil surface.

Locality 2. Terrace surface above South Prong of Little Red River, 1 km northwest of Eagle's Point, Caprock Canyons State Park, Briscoe County, at 710 m elevation; sampled 6 Apr 1984. Soil samples were taken from underneath downed wood or juniper duff in redberry juniper - mesquite (*Juniperus pinchotii* Sudworth - *Prosopis glandulosa* Torrey) scrub. All species in Table 1 under column 2 were found in soil samples taken from underneath downed wood; *Pupoides albilabris* (C. B. Adams) was not found in the sample from juniper duff lacking downed wood (underneath same *J. pinchotii*).

Locality 3. Canyon of East Fork of Rock Creek (Tule Creek drainage), 3.2 km north of Texas 86, 11 km west of Silverton, Briscoe County, at elevation 960 m; sampled 8 Apr 1984. Sample site was a small side canyon off right bank with seeps and abundant leaf litter of plains cottonwood (*Populus sargentii*) over colluvium derived from Pleistocene Tule Formation overlying Triassic sediments. *Vertigo ovata* Say was previously unknown living in study area; only other record of living *E. trochulus* in the study area was Locality 1 (see above). Also significant were the occurrences of the gray slug, *Deroceras laeve* (Müller), and *Gastrocopta abbreviata* (Sterki). *D. laeve* was collected as eggs that later hatched in laboratory.

Locality 4. Hancock Lake, natural lake occupying a subsidence basin (doline), 31 km WSW of Memphis, Hall County, at 623 m elevation; sampled 11 Jul 1984 by S. C. Caran. Living aquatic snails (*Physella virgata*) were collected from depth of less than ten cm; substratum was black, sapropelic (extremely humic), silty to fine sandy clay with strong sulfurous odor. This was first record of living aquatic snails in a natural lake (a doline differs from a playa in geological origin) in study area.

Locality 5. Playa crossed by Texas Highway 217, 3.8 km east of junction with FM 1541, south side of road, Randall County, at 1090 m elevation; sampled 26 Mar 1986 by S. C. Caran. Specimens (all freshwater snails) included first living *Fossaria cockerelli* Pilsbry and Ferriss from the Texas Panhandle. Other species present was *Planorbella tenuis* which has a fine-sculptured shell and is nominally separable from *P. trivolvis*.

Locality 6. Lake Theo, artificial impoundment on Holmes Creek in Caprock Canyons State Park, Briscoe County; two samples.

Sampling of desiccated cattail (*Typha*) marsh along margins of the upper portion of Lake Theo on 5 Oct 1977

revealed two shells of *Oxyloma retusa* (Lea), a species previously not known to live in Texas (elevation - 785 m). Substratum was a reddish sandy silt that is submerged to a depth of approximately one decimeter at normal high water. No shells of other terrestrial gastropods were observed. Shells of *Physella virgata* and *Planorbella trivolvis* were present at this site, indicating recent periods of inundation.

Sampling of benthos by ponar dredge on 10 Aug 1987 by Greg Conley revealed two species of pea clams [*Pisidium casertanum* (Poli) and *P. compressum* Prime] heretofore not reported from the Texas Panhandle. Both species have been reported from Texas by Herrington (1962) who did not provide specific localities. These pea clams were more abundant in the deepest and middle portions of the lake than in the shallow portion, possibly in response to persistent flow of the natural "sweetwater" springs now inundated by Lake Theo, which contains waters with high levels of gypsum. Other freshwater molluscs recovered in these samples included *Gyraulus parvus* (Say), *Physella virgata*, and *Anodonta grandis* (shell fragments only of the latter species); of these species, all but *G. parvus* reported from Lake Theo by Neck (1982).

Locality 7. Turkey Creek (Salt Fork of Red River drainage), 4.8 km northeast of Green Belt Reservoir dam, Donley County, at 798 m elevation; sampled 4 Oct 1987. Sample sites were stream and terrace (underneath downed wood and bark of cottonwood) adjacent to stream, which at this point was a wide pool with associated marsh (*Typha*, *Eleocharis*, *Scirpus*) and localized riparian woodland (*Salix*, *Populus*, *Celtis*, *Cephalanthus*). *Planorbella tenuis* was common in side pools (10-15 m deep) whereas *Physella virgata* and *Fossaria modicella* (Say) were found in shallow pond margins and isolated pools of nearly stagnant water. Terrestrial gastropod fauna of 12 species in riparian soils was exceptionally diverse for the Texas Panhandle. Especially noteworthy was the occurrence of several regionally rare species - *Euconulus trochulus*, *Gastrocopta tappaniana*, *G. abbreviata*, and *Vertigo ovata*. The xeric upland slopes were locally inhabited by *Succinea indiana*.

Locality 8. Bugbee Canyon inlet of Lake Meredith, Canadian River, Hutchinson County, with elevation of reservoir surface at approximately 890 m; sampled 5 Oct 1987 with field assistance by S. C. Caran. This quiet water cove in a reservoir (created in 1965) supported populations of *Corbicula fluminea* (see Neck, 1987b), *Anodonta grandis*, *A. imbecillis* Say, and *Physella virgata*. The three bivalves were previously unknown from the Canadian River drainage in Texas. *A. imbecillis* had not previously been recorded from the Texas Panhandle.

Locality 9. Tule Creek at FM 2301 crossing (Red River drainage), Swisher County, at 988 m elevation; sampled 22 Oct 1987. A *Scirpus/Typha* marsh encircled deeper, open water and was bordered by clay soil with calcareous cobbles. This locality was significant because the permanent water is the result of natural hydrogeological conditions, not human alterations. Especially significant was the occurrence of the finger-

nail clam, *Sphaerium striatinum*, in a natural waterbody (no other such modern records are known).

Locality 10. Buffalo Lake National Wildlife Refuge, Randall County, sediment from currently dry lake (impounded Tierra Blanca Creek of Prairie Dog Town Fork of Red River drainage) and upland soil samples from calcareous sandy soils developed on slopes of Ogallala Formation at 1120 m elevation; sampled 22 Oct 1987. Dominant plants were side-oats grama (*Bouteloua curtipendula* Michaux), little bluestem (*Schizachyrium scoparium* Michaux), sand bluestem (*Andropogon halli*), narrowleaf yucca (*Yucca angustifolia* Hackel), skunkbush sumac (*Rhus aromatica* Aiton), and three-awn (*Aristida* sp.). The freshwater mussel, *Unio merus tetralasmus*, had previously been reported from the Texas Panhandle (Clarke, 1938—specimens in USNM, lot #170791, collected 4 Jul 1937; specimens also collected at Buffalo Lake by A. L. Metcalf 29 Jul 1975, unpublished—MALB lot #49). *Rabdotus dealbatus* (Say) occurred in a tall grassland on slopes; specimens are whitish with faint coffee-colored base (more similar to many specimens of *R. mooreanus* (Pfeiffer) in central Texas than to specimens of *R. dealbatus* from Caprock Canyons State Park - Neck, 1984). *Zonitoides arboreus* (Say) and the other mesic-adapted terrestrial gastropods from this locality were recovered from deep soil, leaf litter, and bark/wood fragments below eastern hackberry (*Celtis occidentalis* Linnaeus) in a wide draw at the floodpool shoreline of the presently dry Buffalo Lake.

Locality 11. Right bank of Prairie Dog Town Fork of Red River, Palo Duro Canyon Park, Randall County, at 875 m elevation; sampled 6 Dec 1988. Sample site was the stream and a terrace with overflow channel between horse stable and park store. Dominated by plains cottonwood, other plant cover included sand bluestem, Indian grass (*Sorghastrum nutans* Linnaeus), and hackberry. Significant was presence of *Gastrocopta armifera* in a xeric site with only large pieces of downed wood and flood debris as cover; no leaf litter accumulations were present.

Locality 12. Free-flowing and impounded portions (Lake Fryer) of Wolf Creek (of North Canadian River); within, upstream, and downstream from Wolf Creek County Park, Ochiltree County, at 795-815 m elevation; sampled 7 Dec 1988. Sampled vegetation communities included hackberry/cottonwood, cottonwood, willow, and Rocky Mountain juniper woodlands. Sites included water courses, extremely mesic stream banks, well-drained slopes, and well-drained terrace. Significant aquatic records included the first records in study area of *Pisidium punctiferum* (Guppy), *P. nitidum* (Jenyns), *Musculium transversum* (Say), and *Fossaria dalli* (F. C. Baker), as well as the first native occurrence of *Anodonta grandis* (as indicated by the occurrence of nested pairs of shells in prehistoric archeological sites). Terrestrial samples yielded first record of *Hawaiia minuscula* (A. Binney) in area, additional records of *Helicodiscus singleyanus* (Pilsbry) and *Euconulus trochulus*, and micro-sympatric occurrence of *Gastrocopta armifera* and *G. abbreviata*.

Locality 13. Drainage of Palo Duro Creek of North Canadian River (within future limits of Palo Duro Reservoir), northeast of Spearman, Hansford County, at 860 m elevation; sampled 7 Dec 1988. At confluence of Palo Duro Creek and Horse Creek, an overmature plains cottonwood riparian woodland with much downed wood on deep sand revealed only *Dero ceras laeve*.

DISCUSSION

COMPLETENESS OF FAUNAL SAMPLING

In general, the report of additional species (see appendix 1) from the Texas Panhandle resulted from surveys of previously unsampled habitats. These newly-sampled habitats had environmental characteristics (physical or biological) that allowed survival of a different suite of species or have zoogeographical dispersal routes available from difference source areas. The only definitely introduced molluscs reported from the Texas Panhandle were one gastropod, [*Rumina decollata* (Linnaeus)] and one bivalve (*Corbicula fluminea*). Undoubtedly, other introduced gastropods were established in urban areas, but these habitats have not been sampled adequately at this time (but see Neck, 1989a). The populations of *Oxyloma retusa*, *Anodonta imbecillis*, and *Pisidium compressum* should be considered recent introductions via migratory waterfowl until native populations in natural habitats, i.e. non-reservoir sites, are located. These species are not native in the strictest sense of the term, but neither have they been introduced deliberately or even indirectly by human activities. The habitats required by these aquatic and amphibious species are not represented in the Texas Panhandle except in areas modified by human activity.

Sampling of additional sites with dependable, i.e. permanent, water supplies, and/or protection from intense summer solar insolation could reveal additional species to be living in this area, although "the number to be expected is very low" (Neck, 1984). However, certain habitats have been sampled sufficiently that additional species are unlikely to be recovered from these areas. The xeric scrub in the "badlands" along the eastern margin of the Caprock Escarpment is a harsh environment for land snails. Dominated by mesquite and red-berry juniper, this community is characterized by limited humus development, exposure to extremes of temperature, and susceptibility to soil erosion. Collections at Caprock Canyons State Park (locality 2) revealed that several species of land snails can survive in the limited humus available under junipers in the absence of downed wood. However, only species previously reported from this habitat were recovered.

Additional species are known from areas immediately south of the Texas Panhandle. Sublette and Sublette (1967) reported *Fossaria bulimoides* from three to nine playas surveyed on the Southern High Plains of Texas and New Mexico south of the present study area (but see reference to this taxon in subsection on Nomenclatural and systematic compilations). Fullington (1978) reported the tropical/semiotropical aquatic snail *Biomphalaria havanensis* (Pfeiffer, 1839) from Garza and Scurry counties in the southern Rolling Plains.

Pierce (1975) reported several "modern faunas" from drift samples from Lynn County, south of the Texas Panhandle. Two species not known living in the Texas Panhandle were listed from these samples. *Pupilla blandi* E. S. Morse ("dried flesh in several shells") and *Vertigo gouldi* (A. Binney) ("very fresh and unweathered") were allegedly documented as living. Populations of these two species as well as species known from adjacent counties of adjoining states could also occur in the Texas Panhandle.

Although only limited records of molluscs had been reported in the Texas Panhandle prior to the current series of field studies, additional surveys have been accomplished in areas north of the Texas Panhandle. Surveys of several counties in southwestern Kansas have indicated the occurrence of significant but low-diversity molluscan faunas (Leonard, 1943; Branson, 1972). Several additional species not known to live in the Texas Panhandle have been reported living in Cimarron County, in the Oklahoma Panhandle (Metcalf, 1984). Three species—*Gastrocopta holzingeri* (Sterki), *Pupilla muscorum* (Linnaeus), and *Pupoides inornatus* Vanatta—are known from basaltic talus (a microhabitat not present in the Texas Panhandle) and may owe their occurrence in Cimarron County to periodic immigration from upstream populations in the Sangre de Cristo Mountains of northern New Mexico and southern Colorado, or in basalt fields of northeastern New Mexico.

The apparent absence of at least one freshwater gastropod from the Texas Panhandle is significant. *Helisoma anceps* (Menke) is a common species in Pleistocene fossil assemblages from this area. Living populations of *H. anceps* are known from the Hill Country of Texas on rocky substrates (bedrock or gravel). This habitat is not present in the Texas Panhandle at present. Elsewhere in North America, *H. anceps* may be found in lakes, ponds, rivers, and streams with vegetation on various substrates in permanent water (Clarke, 1981). Sufficient areas of this habitat do not occur in the Texas Panhandle today; otherwise *H. anceps* could be present.

NOMENCLATURAL AND SYSTEMATIC COMPLICATIONS

Even if faunal sampling efforts were considered to be reasonably complete, ecological analysis of the fauna is dependent upon the state of systematic and nomenclatural knowledge and stability. The application of proper scientific names to certain molluscan taxa of the Texas Panhandle is complicated by a lack of detailed taxonomic knowledge. The general uncertainty associated with proper identification of taxa of the family Succineidae has been mentioned earlier, as has the close relationship between *Planorbella trivolvis* and *P. tenuis*. Also unclear is the separate status of *Fossaria cockerelli* (listed in Table 1) and *F. bulimoides* (mentioned in previous subsection). Part of the doubt concerning these latter two pairs of species is caused by changes in perceived systematic status through time. Analysis of published records of these taxa is complicated because of the unavailability of reference specimens.

Very few records of modern *Gastrocopta abbreviata*

have been reported from Texas. The original record by Pilsbry (1948) has been suspected (and rightly so) of being a fossil drift specimen (Cheatum and Fullington, 1973). Hubricht (1985) listed a record from Potter County. The relationship of *G. abbreviata* to its very closely related congener, *G. armifera* (Say, 1821) has been unclear, although Hubricht (1972) demonstrated slight but distinctive differences between these two forms. Turkey Creek (locality 8) was the first reported micro-sympatric occurrence of living populations of these two species in Texas, although co-occurrence in fossil assemblages in central Texas was reported by Hubricht (1972). Co-occurrence of living *G. abbreviata* and *G. armifera* has been reported in Missouri, Mississippi, Oklahoma, and Kansas (Hubricht, 1972).

Subsequent systematic studies could alter the perceived relationships of some or all of these closely related taxa. Such changes could alter the exact number of species recognized in total or within any of several ecological communities. However, the environmental settings of these populations are now known. Therefore, an ecological analysis of the modern native molluscan fauna of the Texas Panhandle is presented below.

MOLLUSCAN COMMUNITIES OF TEXAS PANHANDLE

The major factor controlling species diversity of living molluscs in the Texas Panhandle is reliable water. A dependable water supply provides a medium in which aquatic gastropods and bivalves can live. Additionally, water also allows development and maintenance of trees that produce leaf litter, downed wood, and shade, which conserve soil moisture and ameliorate the devastating effects of direct solar exposure upon terrestrial gastropods.

Several community types of aquatic molluscs can be recognized in the Texas Panhandle. Perennial creeks without excessive loads of dissolved solids are occupied by *Gyraulus parvus*, *Planorbella tenuis*, *Fossaria cockerelli*, and *Hebetancylus excentricus*. Playas and stockponds typically have *Physella virgata*, *P. trivolvis* (and occasionally *P. tenuis*), and *G. parvus* (latter species typically restricted to certain playas). Intermediate and large reservoirs contain many of the above species in protected coves, but also can contain the bivalves *Anodonta grandis*, *A. imbecillis*, *Corbicula fluminea*, and *Sphaerium striatinum*. Several species of pea clams (*Pisidium* spp.) are known from this area, but the limiting ecological factors are not understood.

A predictable suite of terrestrial gastropods is characteristic (but not restricted to) upland, well-drained microhabitats that represent the most extreme xeric conditions that are tolerated by terrestrial gastropods in the Texas Panhandle. The fauna of this upland community consists of varying proportions of the following species: *Pupoides albilabris*, *Gastrocopta procera* (Gould), *G. pellucida* (Pfeiffer), *G. cristata* (Pilsbry and Vanatta), and *Helicodiscus inermis* H. B. Baker. These species can withstand lengthy periods of drought with minimal cover. Thick leaf litter suffices as minimal cover for all of the above species except for *P. albilabris* which requires additional cover in the form of downed wood.

The more mesic-adapted terrestrial gastropods of the Texas Panhandle can be readily divided into two communities. The more drought resistant of these species (*Vallonia parvula* Sterki, *V. gracilicosta*, and *Gastrocopta armifera*) survive in well-drained habitats if sufficient cover in the form of rocks, downed wood, or thick leaf litter occurs at the site. These species are adapted to microhabitats that conserve maximum percentages of the moisture supplied by local precipitation, including that falling immediately upslope. The less drought-resistant of the mesic-adapted species (*Vertigo ovata*, *Euconulus trochulus*, *Gastrocopta tappaniana*, and *Deroceras laeve*) are able to survive drought periods only in microhabitats which obtain moisture input in addition to local precipitation. Such additional soil moisture could represent ground-water seepage or floodplain wicking of surface stream water. Often both of the above communities occur together in bottomland habitats with additional moisture input.

PLAINS COTTONWOOD AS A HABITAT INDICATOR

The downed wood that is a major moisture conservation agent for terrestrial gastropods in the Texas Panhandle is almost invariably from plains cottonwood, *Populus sargentii* Dode, or common hackberry, *Celtis occidentalis*. *P. sargentii* is the most abundant source of downed wood in streamside habitats. Although willows, *Salix* sp., are present along some streams, downed wood from willow often occurs in the stream and does not provide suitable habitat for terrestrial gastropods. *C. occidentalis* normally occurs at a slightly higher elevation above the stream than *P. sargentii*, but the former species supplies downed wood, which provides sufficient cover for most of the more drought resistant species of the mesic-adapted fauna.

Populus sargentii is not an absolute indicator of the existence of the most mesic-adapted gastropod community, because it can occur in well-drained sites that support only the xeric-adapted gastropod community; Neck (1984) noted occurrence of only *Pupoides albilabris* and *Gastrocopta cristata* (Pilsbry and Vanatta) at locality B-1, a site on the floodplain of an intermittent stream, the South Prong of the Little Red River, which exhibits high variation in stream flow with minimal surface water for the major part of the year. At this site, the soil is typically desiccated allowing the existence of a low diversity terrestrial gastropod fauna that contains only the xeric-adapted species. Only a slug, *Deroceras laeve*, which is able to burrow deeply to subterranean moisture sources, can survive in the deep sands at locality 13 even though a thick stand of *P. sargentii* and much downed wood exists at this site.

Populus sargentii is also the dominant tree species on floodplains of large streams such as the Canadian River. At these sites, large trees exist in a parkland community with only limited understory vegetation. No molluscan samples have been obtained from such large-stream floodplain environments. Surface water levels in such habitats are extremely variable—from absent to high (during massive flood events).

The mesic-adapted terrestrial gastropod community appears to require a sufficient depth of sandy soil to provide

a subsurface "moisture bank." Terrestrial gastropod faunas with the greatest percentage of mesic species exist in *Populus sargentii* woodlands on floodplains of perennial creeks which have constant, high levels of soil water. Sites of this type (Turkey Creek at locality 7 in this study) support a high-diversity fauna that contains most of the living terrestrial gastropods of this region except for those that require well-drained soils. Sites that are characterized by low-volume but rather constant soil water levels, e.g. the seep at locality 3 of this study, support a moderate-diversity fauna dominated by mesic-adapted species.

The germination requirements of *Populus sargentii* include bare or disturbed sand or fine gravel in full sunlight with few or no plant competitors (Read, 1958; Everitt, 1968). Survival of seedlings and saplings is dependent upon shallow ground water. Old trees could survive some distance from the current stream location as long as the increased relative elevation (resulting from downcutting of the stream) does not preclude tapping of shallow ground water by the root system.

Terrestrial gastropods, however, are dependent upon the presence of cover objects (rock, wood, leaf litter, etc.), which extend the time period that soil moisture levels are conducive to gastropod activity. Soil moisture levels in the Texas Panhandle are adequate below seeps and on low floodplains where surficial flow and subsurface wicking exists. High floodplain flats and sandbars do not normally contain high soil moisture levels. Although large *Populus sargentii* can survive on ground water, terrestrial gastropods must have high surface soil moisture levels. The direct importance of soil moisture and cover objects is well illustrated by the occurrence of several mesic-adapted terrestrial gastropods on the floodplain of the Washita River (locality 1, this study) in the absence of woody vegetation but with scattered pieces of downed wood.

FUTURE STUDIES

The finding of a substantial number of additional living species of molluscs in the Texas Panhandle raises questions as to the validity of previous environmental reconstructions based upon molluscan faunas as then known (Pierce, 1975; Neck, 1978; Johnson *et al.*, 1982; Neck, 1987a). These questions must be addressed if such reconstructions are to be considered accurate. Increased knowledge of the molluscan fauna of the area when combined with faunal surveys of adjacent areas will allow an improved interpretation of the zoogeography of the living molluscs of the Texas Panhandle.

ACKNOWLEDGMENTS

I thank S. Christopher Caran and Greg Conley for supplying several collections utilized in this study. Christopher B. Beckcom and Steven Gaddy provided drafting assistance. S. C. Caran also reviewed an earlier version of this manuscript. Several operators of the Information Services Section of Texas Parks and Wildlife Department produced several manuscript versions of this paper.

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Date of manuscript acceptance: 17 May 1990.

Appendix 1.

ADDITIONAL SPECIES RECORDS

Fossaria cockerelli (Pilsbry and Ferriss) occurs sporadically in intermittent water through most of the United States west of the Mississippi River (Hibbard and Taylor, 1960). Records of living snails in Texas were known only from Hunt and Fayette counties (Fullington, 1978:143), no closer than 550 km to this Randall County record, the site of the present record. However, New Mexico populations, including the type locality, are much closer to the Texas Panhandle (Bequaert and Miller, 1973). These Randall County specimens are referred to *F. cockerelli* by comparison to available drawings and photographs (Pilsbry and Ferriss, 1906; Fullington, 1978; Burch, 1982). Taxonomic relationship of *F. cockerelli* to *F. bulimoides* (L. Lea) has been unclear, but the occurrence of sympatric, non-transitional populations in Arizona (Bequaert and Miller, 1973: 197) indicates that these taxa are

distinct at the species level. Fossil shells of the *F. bulimoides* group from the Texas Panhandle have been referred to both *F. cockerelli* (Frye and Leonard, 1957) and *F. bulimoides* (Taylor, 1960; Hibbard and Taylor, 1960).

Fossaria dalli (Baker) ranges from southern Canada southward to Arizona and Texas (Fullington, 1978; Clarke, 1981). Texas records are few in number but range from the humid Coastal Plain of east Texas to the Chihuahuan Desert of West Texas (Fullington, 1978). *F. dalli* is known from sites with vegetation and variable substrates in marshes, ponds, lakes, and small rivers (Clarke, 1981).

Fossaria modicella (Say) ranges from Nova Scotia to Vancouver Island southward to Alabama and southern California (Baker, 1928; Taylor, 1987). Fullington (1978) recorded no records from Texas of living *F. modicella*, a species that he synonymized under *F. obrussa* (Say) which was widespread in Texas during the Pleistocene (but not reported as living in Texas). Preferred habitats include perennial ponds, streams, and lakes as well as vernal ponds (Clarke, 1981).

Fossaria parva (Lea) has been recorded from a large geographic area from Connecticut to Alberta southward to Maryland, Oklahoma, and Arizona (Burch, 1982; Taylor 1987). Fullington (1978) reported widespread fossil occurrences but no living records of this species, which he synonymized under *F. obrussa*. *F. parva* is an amphibious species which has been reported from marshes, mud flats, lakeshores, and occasionally shallow water (Clarke, 1981).

Hebetancylus excentricus (Morlet) ranges from Central America to Texas and Florida (Basch, 1963). No previous records of living freshwater limpets (family Ancyliidae) are known from the Texas Panhandle (see Fullington, 1978). The closest living population in Texas was reported from Palo Pinto County (approximately 400 km to the southeast) as *Gundlachia radiata* (Guilding) in Fullington (1978). *H. excentricus* is not known from any fossil fauna in Texas (Fullington, 1978) nor is it known from Oklahoma (Branson, 1961) or Kansas (Leonard, 1959), indicating that this species has probably increased its range northward since the Late Pleistocene as water temperatures warmed. Whether the recent increase in known localities in north central Texas (McMahon and Aldridge, 1976) is due to a warming climate, increasing percentage of lotic (vs. lentic) waters, or increasing survey efforts is unknown. Preferred habitat is rock or wood substratum flowing or still waters (Fullington, 1978).

Gastrocopta tappaniana (Adams) ranges from Ontario to Maine to Alabama, Texas, Kansas, Arizona, and South Dakota. *G. tappaniana* is found in moderately mesic riparian woodlands of the Coastal Plain in eastern and central sections of Texas (Cheatum and Fullington, 1973). Presence at locality 1 indicates that ground cover and soil moisture are the primary habitat requirements. These requirements are generally provided by downed wood and leaf litter under deciduous trees, but trees may not be a primary requirement. Hubricht (1985:76) recorded *G. tappaniana* from Beckham County, Oklahoma, approximately 85 km southeast of locality 1.

Vertigo ovata (Say) ranges over most of northern and eastern North America from the Aleutian Islands to Labrador southward to Florida and eastern Texas; southwestern limit of its range is formed by Nebraska, Colorado and Arizona (Bequaert and Miller, 1973:183). Valid Texas records are known from eastern Texas, where it is "associated with considerable moisture," as far west as Dallas County (Cheatum and Fullington, 1973). Closest known living populations are in Beckham County, Oklahoma, approximately 190 km northeast of locality 3 (Hubricht, 1985). Bequaert (in: Bequaert and Miller, 1973) found "no reliable evidence" of living populations in the Texas Panhandle after collecting over a four year period. In the southwestern portion of its range, *V. ovata* is extremely sporadic, possibly due to chance transport via waterbirds (Bequaert and Miller, 1973). Fossil records of *V. ovata* from the Panhandle are common (Schultz and Cheatum, 1970; Johnson *et al.*, 1982; Neck, 1987a).

Oxyloma retusa (I. Lea) ranges from New England west to North Dakota and southward to Virginia and New Mexico (Metcalf in Bequaert and Miller, 1973:155; Hubricht, 1985). Typical habitat for this species includes riverbanks, pond margins, marshes, and swamps (Calkins in: Pilsbry, 1948; Leonard, 1959; Frest and Dickson, 1986). Previously unrecorded from Texas, *O. retusa* has been reported from Oklahoma and New Mexico (Branson, 1962; Metcalf in: Bequaert and Miller, 1973:155). Southern Great Plains populations of *Oxyloma* have been referred to *O. haydeni* (W. G. Binney) by some workers (Harris and Hubricht, 1982; Hubricht, 1985).

Helicodiscus singleyanus (Pilsbry) is known from various open habitats from scattered localities from New Jersey to New Mexico and Arizona (Bequaert and Miller, 1973; Hubricht, 1985). Many published records of *H. singleyanus* from the southeastern United States refer to other species of the subgenus *Hebetodiscus*, including *H. inermis* which is also known from the Texas Panhandle.

Hawaiiia minuscula (A. Binney) has a native range that covers much of the Western Hemisphere from Newfoundland to Alaska south to Central America and the West Indies; subsequently, this species has become almost cosmopolitan due to spread by human activity (Bequaert and Miller, 1973). Leonard (1959) reported *H. minuscula* under rocks and wood as well as in grass clumps in both bottomland and upland environments.

Zonitoides arboreus (Say) ranges throughout the United States from coast to coast, although there is a large gap in the Great Plains where the lack of trees provides few areas of suitable habitat. The nearest records to Buffalo Lake National Wildlife Refuge are Cimarron County, Oklahoma; Wichita County, Texas; and San Miguel County, New Mexico (Hubricht, 1985). In the southwestern United States, *Z. arboreus* is native at higher elevations (up to 3650 m); below 1525 m it occurs generally only as temporary adventive populations (Bequaert and Miller, 1973:88). *Z. arboreus* is a common urban species (Hubricht, 1985:32), including the Southern High Plains of Texas (Neck, 1989a).

Euconulus trochulus (Reinhardt) has a broad geographic range from southern Canada to the Gulf coast westward to the Great Plains. This taxon has long been considered a subspecies of *E. chersinus*

(Say), but Hubricht (1983) raised *trochulus* to specific status. Texas records are generally from the Coastal Plain (Hubricht, 1985). In the Texas Panhandle, *E. trochulus* lives in narrow, protected canyons with leaf litter and moist sandy floodplains with protective cover.

Anodonta imbecillis Say ranges from southern Ontario to Georgia and westward to the northern Great Plains and northern Mexico (Clarke, 1981), where it is found in a variety of waterbodies from ponds to small rivers and lakes on mud or sand substrates (Clarke, 1981; Neck, unpub. data). Native range in the Red River of the South extends as far west as the Wichita Falls, Wichita County area (Neck, 1989b). Occurrence of *A. imbecillis* in Lake Meredith (locality 8) is due to human introduction.

Musculium transversum (Say, 1829) ranges from Quebec to the Northwest Territories southward into Mexico (Clarke, 1981). Usually found in mud (but occasionally in sandy substrata), *M. transversum* is known from sloughs, lakes, and rivers (Herrington, 1962). I have collected this species in central Texas usually from moving water or ponds with freshwater input (Neck, unpub. data).

Pisidium casertanum (Poli) is found in almost all of the Western Hemisphere, ranging from Alaska to Patagonia (Hibbard and Taylor, 1960). This species inhabits lakes, ponds, streams, swamps, and even temporary water habitats (Herrington, 1962; Clarke, 1981).

Pisidium compressum Prime ranges over most of North America from the Northwest Territories southward through the United States to Mexico (Herrington and Taylor, 1958). *P. compressum* can be found in lakes, rivers, and creeks but never in swamps, lagoons, or bog ponds (Herrington and Taylor, 1958), although Clarke (1981) stated that its usual habitat is in shallow water among vegetation.

Pisidium nitidum Jenyns has a Holarctic range and is found over most of North America except for the southeastern United States (Hibbard and Taylor, 1960). *P. nitidum* can survive in almost any perennial body of water, but is usually found in shallow water (Clarke, 1981).

Pisidium punctiferum (Guppy) ranges from southern Canada to Uruguay with additional records from Europe (Herrington, 1962). *P. punctiferum* is known from creeks, rivers, and lakes according to Herrington (1962), who reported this species from unspecified locations(s) in Texas.

MATURATION OF THE REPRODUCTIVE TRACT OF *NEOHELIX MAJOR* (BINNEY) (GASTROPODA: POLYGYRIDAE)

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ABSTRACT

Maturation of the reproductive system of *Neohelix major* (Binney), from post-hatching through sexual maturity, was examined. Laboratory reared snails were used throughout this study so that ages of animals were known. Snails were sampled every 30 days starting at one month and continuing through 14 months of age. The ovotestis, hermaphroditic duct, albumen gland, spermoviduct and terminal genitalia (free oviduct, vas deferens, vagina, penis and bursa copulatrix) were examined histologically.

Considerable intraspecific variation exists in the structural development of the reproductive tract of *Neohelix major* even among animals of the same age. Within the same adult age class, animals with shells in good condition showed uniformity in development but individuals with damaged shells were variable and always immature. There is a relationship between shell diameter and degree of maturity reached by the reproductive tract. Snails four months old contained spermatogonia and spermatocytes, but mature spermatozoa did not appear until the snails were nine months old. Oocytes were present from the first month although the largest appeared at 12 months of age. Until eight months of age the reproductive tract appeared immature and male organs were more evident. At nine months old, all organs, especially those of the female, increased in size and became more voluminous. The male system completed maturation slightly earlier than did the female system. Maturity is reached for most of the animals between 10 to 12 months of age when the ovotestis already contains spermatozoa.

The reproductive anatomy of several Tridopsinae has been studied by Simpson (1901), Pilsbry (1940) and Emberton (1988). Although the biology of *Neohelix major* (Binney) has been studied by Vail (1978) during two consecutive reproductive seasons, details of the changes in the reproductive system from hatching to sexual maturation are not yet available for this species. In contrast, detailed descriptions are available for *Arion ater rufus* Linné based on material reared in the laboratory (Lusis, 1961) and collected from the field (Smith, 1966), and also for *Agriolimax reticulatus* (Muller) (Runham and Laryea, 1968; Runham, 1978).

Other authors have described various aspects of maturation of the reproductive tract and suggested the possibility of controlling factors. Laviolette (1954) described the role of the gonad in the maturation of the reproductive tract in the Arionidae suggesting hormonal control. Smith (1966) found a close relationship between maturation and seasons, and later (1967) investigated the relationship between neurosecretory activity and maturation.

This paper describes different stages in the maturation of the reproductive tract of the polygyrid gastropod *Neohelix major*, from recently hatched snails to maturity.

MATERIALS AND METHODS

This study was based upon snails reared in the laboratory by Dr. Virginia Vail. The parental stock comprising 21 mature specimens came from Greenwood Plantation, near Thomasville, Thomas County, Georgia, U.S.A. Snails were maintained under laboratory conditions over two consecutive reproductive seasons. Vail made observations on the eggs and young (Vail, 1978), and fixed snails of known ages during the summer season (June-July) so it was possible to compare not only animals of different ages but also individuals of the same age. Following a baseline study of these preserved snails, animals of known ages were sampled every 30 days, starting at one month and continuing until 14 months of age.

Generally, each sample consisted of five to seven snails but some critical ages necessitated analysis of more snails. Individuals of each age-class belonged to the same clutch of eggs. Before dissecting the animals, shell diameter and shell height were measured.

Snails of each sample were dissected and ovotestis, hermaphroditic duct, albumen gland, spermoviduct, free

oviduct, vas deferens, penis, vagina and spermatheca were embedded in paraffin blocks, sectioned at 6 and 10 μm , and stained with hematoxylin-eosin or Feulgen-fast green.

In the case of younger snails, when it was not possible to dissect out certain organs, the whole animal or portions of it were embedded and sectioned. Entire structural tracts of different ages were drawn at the same magnification with the help of a camera lucida to observe qualitative differences with respect to the size of the organs. Quantification of the differences have not been practical because of the irregular shape of the organs.

RESULTS

GROSS ANATOMY

In sexually mature individuals of *Neohelix major* (Fig. 1), the ovotestis is embedded in the digestive gland. Small collecting ducts join in sequence to form the hermaphroditic duct which, at first, is narrow and straight proximally, then becomes wide and strongly coiled, finally again narrowing distally. This duct ends in the talon at the base of the large, curved albumen gland. The duct of the albumen gland joins the spermoviduct which is a long and convoluted organ that consists of incompletely separated female and male parts. At its distal portion the spermoviduct splits into two separate ducts: the vas deferens and the free oviduct. The vas deferens is a long, coiled narrow duct that continues to the proximal part of the penis, whose walls are thick and muscular. The free oviduct is short and ends by joining the spermathecal duct to form the vagina. The vagina and penis have a common gonopore located on the right side of the head.

OVOTESTIS

When fully developed the ovotestis consists of many lobes, each consisting of numerous tubules. Their number is variable and related to the size and age of the specimen.

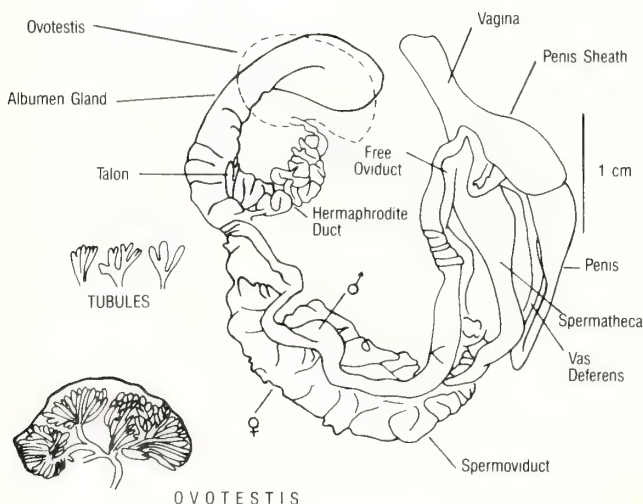


Fig. 1. Gross anatomy of the reproductive tract of *Neohelix major*.

The tubules are connected via small collecting ducts to the main hermaphroditic duct. At first, these tubules are short, but later increase in size to form longer sacs. Usually the tubules are simple but sometimes they bifurcate or trifurcate. Both male and female gametes develop in the same tubule.

Four general stages are recognized in the ovotestis: a) undifferentiated; b) spermatocyte; c) spermatid; d) spermatozoa. The undifferentiated stage extends from the first through the fourth month of life. According to Luchtel (1972), at first two types of cells form the gonad: germinal and non-germinal cells. The germinal line differentiates into either spermatogonia or oogonia and the non-germinal cells give rise to follicle and sertoli cells, the so-called "auxiliary cells" (Fig. 2). Morphological differences among germinal and non-germinal cells are difficult to distinguish with light microscopy, but it appears that non-germinal cells are peripherally positioned and germinal cells are centrally positioned in the young gonad. Also, peripheral cells are smaller and stain more deeply with hematoxylin than central ones.

At one month of age tubules are very small and almost round. By two to three months of age more tubules are visible embedded in different portions of the digestive gland; they have increased in size, mainly in length, and are completely filled with germinal cells. The spermatogonia have a small amount of cytoplasm in comparison to the size of the nucleus. Their nuclei are usually granular in appearance.

SPERMATOCYTE STAGE

The spermatocyte stage seems to extend over a considerable period of time. When snails are four months old, the tubules are densely packed, and the first spermatocytes and oocytes can be distinguished (Fig. 3). Spermatocytes have more cytoplasm than spermatogonia and the nucleus is bigger and less granular. Spermatocyte nuclei are spherical or oval in shape. Different stages of meiosis are seen among them. Usually they are placed in the lumen of the tubule while oocytes are always related to auxiliary cells in the tubule epithelium. Oocytes have nuclei with peripherally located nucleoli. They occur in groups of two to four, although they can be found alone. The tubules increase in number and size through five to seven months of age. During these months spermatocytes have also increased in size and number. Groups of them are interconnected by cytoplasmic bridges and attached to Sertoli cells located in the tubular epithelium. Spermatocytes are especially abundant when the snails are five to ten months old. At the age of eight months some oocytes show indications of vitellogenesis.

SPERMATID STAGE

The spermatid stage is reached by nine months, when the number of spermatocytes have increased considerably. Secondary spermatocytes are identifiable. Large numbers of dividing cells showing various meiotic figures are characteristic of this stage. Several stages of spermatid differentiation were observed. In early stages much cytoplasm surrounds the nucleus, which is round and compact (Fig. 4). At this point

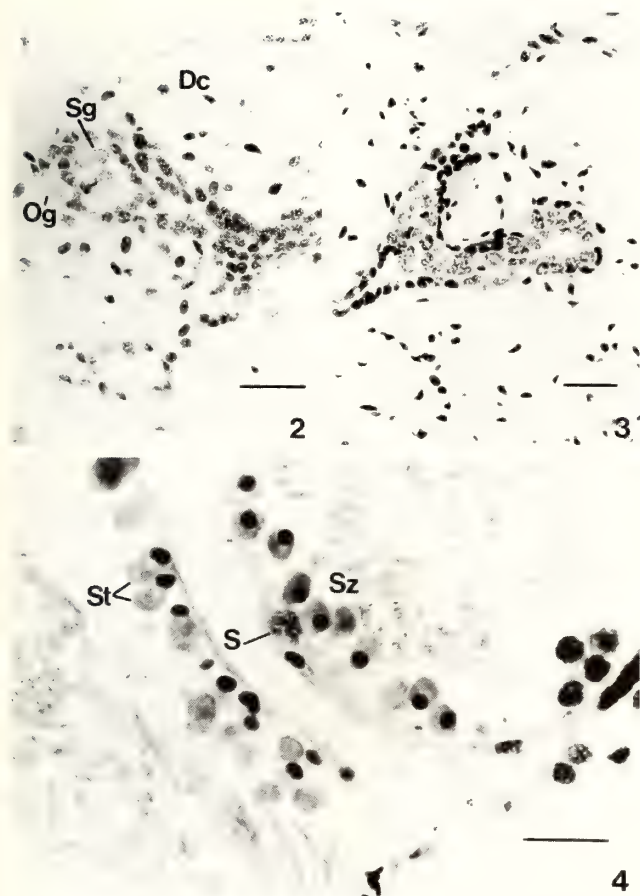


Fig. 2. Undifferentiated stage: tubule (at two months of age) showing germinal cells centrally positioned, one of them in metaphase, and non-germinal cells peripherally positioned (Dc, digestive cells; Og, oögonia; Sg, spermatogonia) (scale bar = 40 μ m). **Fig. 3.** Spermatocyte stage: bifurcate tubule (at four months of age) embedded in the digestive gland. The first spermatocytes and oocytes can be distinguished (scale bar = 40 μ m). **Fig. 4.** Spermatid stage: early spermatids can be distinguished in the ovotestis of snails at nine months or older. The nucleus has migrated to the periphery of the cell (St, spermatids; S, Sertoli cell; Sz, spermatozoa) (scale bar = 30 μ m).

it can be difficult to differentiate secondary spermatocytes from early spermatids. Later, the nucleus migrates toward the periphery of the cell and the mass of cytoplasm is found behind the head of the spermatid. Then a depression is formed at the base of the nucleus and in some cases it is possible to see a flagellum beginning to form. In later stages the head and the cell itself become elongated. As the tail grows in length the mass of cytoplasm becomes thinner until only a remnant of cytoplasm remains in the last portion of the tail (Fig. 5).

The first spermatozoa appear by nine months, although they are not very abundant. No spermatozoa, however, were present in the hermaphroditic duct at this time. It is possible that a certain quantity of spermatozoa need to be formed before they are transferred to the acinar ductules.

SPERMATOZOA STAGE

At the age of ten months, sperm start to be released into the hermaphroditic duct. The cytoplasm of the mature spermatozoa is completely sloughed off, and the head is long and curved. The head stains with uniform intensity. The mature sperm are attached mainly in clumps to large Sertoli cells. At this time the ovotestis is large with thin walls. At 13 months of age, the spermatogenic layer is thinner and some tubules appear empty, while others still have a large number of spermatozoa. Spermatocytes are few and spermatids are present in different stages of differentiation. By 14 months of age, the diameter of the tubules has decreased, as much sperm has passed out to the hermaphroditic duct. The oocytes are large and stain lightly.

HERMAPHRODITIC DUCT

The hermaphroditic duct is a long, strongly coiled duct when fully developed. It is narrow at the proximal part when it leaves the gonad and then becomes wide but narrows again at the entrance of the proximal part of the albumen gland. Great changes in the diameter of the duct occur at different ages. Before five months of age it is a straight and narrow duct, and it shows the first signs of coiling at the spermatocyte stage, at five to seven months. During the spermatozoan stage (10-12 months) it reaches its full development and becomes thick and highly coiled.

The hermaphroditic duct is made up of a layer of ciliated, columnar epithelial cells surrounded by a thin muscular layer. A third layer of connective tissue covers the muscular one.

At ten months of age sperm formed in the ovotestis begin to be liberated into the hermaphroditic duct, which increases in diameter and appears to have thinner walls. All animals examined that were ten months or older and whose gonads were at the spermatozoa stage, had sperm in the hermaphroditic duct.

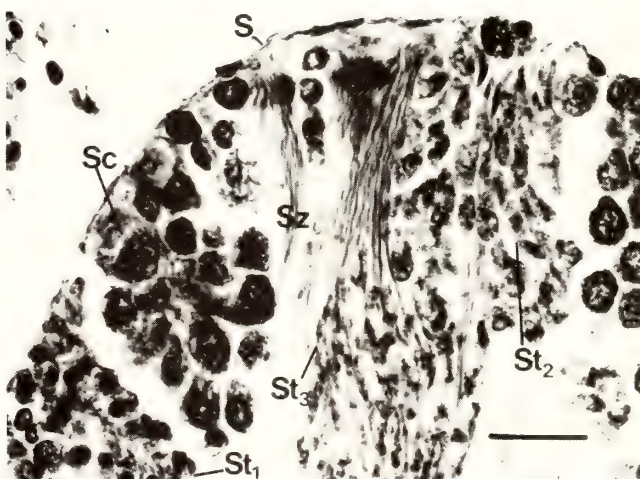


Fig. 5. Several stages of spermatid (St) differentiation in section tubule (at ten months of age). A group of spermatocytes (Sc) is attached to a Sertoli cell (S) (scale bar = 40 μ m).

ALBUMEN GLAND

The albumen gland is a cylindrical organ, curved in adults, which provides the perivitelline fluids for the eggs (Rudolph, 1980). It is organized into secretory follicles that are circular in cross section. Each follicle possesses a small central duct surrounded by a single layer of columnar cells. At the base of the albumen gland exists a structure called "talon or accessory gland" by Simpson (1901), which is the "fertilization pouch" that Lind (1973) described for *Helix pomatia*. This appendage is a digitiform sac that connects with the hermaphroditic duct.

Prior to age five months, the albumen gland is a small organ consisting of a few follicles, then during the 7th and 8th months it begins to increase in size and more follicles are present.

The duct of the talon joins with the albumen gland's duct at the place where the spermooviduct starts. There appears to be direct communication between the spermooviduct and the groove of the talon so the sperm would not enter the oviduct groove. By 12 months of age, the albumen gland is voluminous. The secretory cells have a prominent cytoplasm in which the secretion is stored.

SPERMOVIDUCT

The spermooviduct is a glandular structure whose thickness varies with age and developmental stage of the animal. At its distal portion the spermooviduct splits into a short, free oviduct and a longer coiled vas deferens. This separation into two free ducts occurs during or shortly after the first months of the animal's life and is completed by four months of age. The spermooviduct is made up of two major components: the female and male portions. These portions are parallel to each other and are incompletely separated by longitudinal folds. The female component is composed of a wide duct lined by a thin cuboidal epithelium bearing short cilia. There are two types of glandular tissues associated with the female duct. The main one is formed by large gland cells opening individually into the lumen of the female duct via short ducts between the epithelial cells (Fig. 6). This tissue forms the characteristic folds of the spermooviduct, which are pronounced in the middle part of the organ. When glandular cells begin to produce secretion that fills their cytoplasm, they undergo a large increase in volume and the organ becomes quite thick. It is possible then to see many secretory granules liberated in the lumen of the female duct. The other female glandular tissue is composed of smaller cells and it is located at the junction of the albumen gland and the spermooviduct (Fig. 7). In 10 month old animals the cells are filled with eosinophilic secretions. According to Runham (1978) these glands could either add water to the albumen gland secretion or form the thin layer that surrounds the perivitelline fluid.

The male component of the spermooviduct is formed by a thin duct and there are three types of glands associated with it (Fig. 8). The sperm duct is much narrower and less folded than the female duct and is lined by a columnar epithelium bearing long cilia that form a thick stratum. Transport of sperm is probably carried out by ciliary action

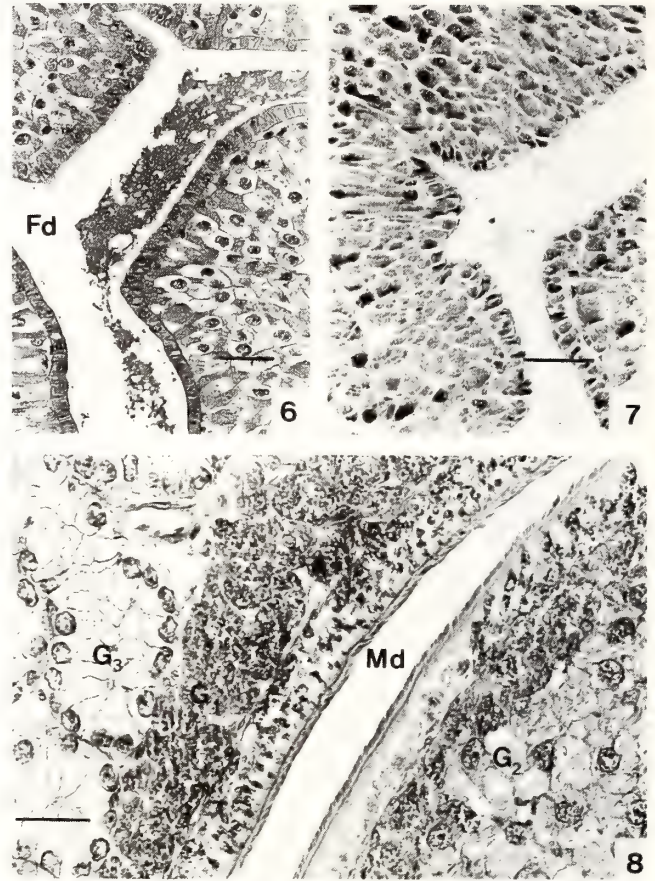


Fig. 6. Section of the female portion of the spermooviduct showing the female duct lined by a cubical epithelium bearing short cilia. This type of tissue forms the characteristic folds of the organ (Fd, female duct) (scale bar = 40 μ m). **Fig. 7.** Female glandular tissue of the spermooviduct located at the junction with the albumen gland (scale bar = 40 μ m). **Fig. 8.** Male part of the spermooviduct formed by a thin duct and the three types of glands associated with it (Md, male duct; G, glandular tissue) (scale bar = 40 μ m).

of the epithelium. Surrounding the duct there is a gland made up of small cells which open to the lumen individually by short ducts that pass through the epithelium. Surrounding this gland there is a second type of gland. It is composed of larger cells, with individual ducts, whose cytoplasm contains large secretory granules with eosinophilic secretions. This gland is always situated between the first one and the prostate gland.

The prostate is the largest and most complex gland of the male part and is situated external to the others. It is organized into secretory follicles formed by cells radially arranged around a central lumen. The ducts of these follicles are long and discharge secretion into the lumen of the sperm duct.

The spermooviduct in *Neohelix major* does not show much differentiation until four months of age, at the beginning of the spermatocyte stage. A common duct is present, lined by a compact epithelium. The other identifiable part is the prostate gland which already has a few follicles (Fig. 9).

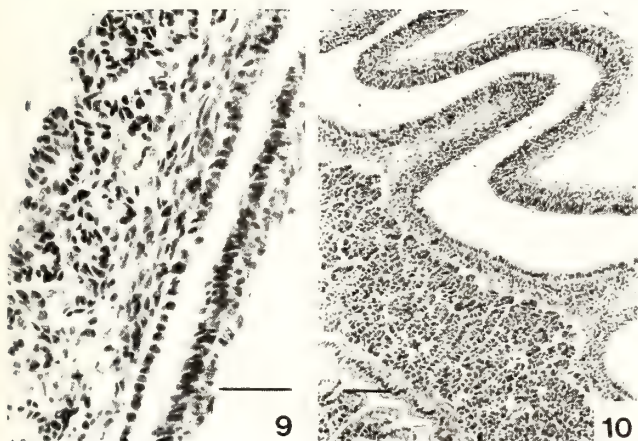


Fig. 9. Longitudinal section of spermoviduct in a five month old animal (scale bar = 40 μ m). **Fig. 10.** Longitudinal section of spermoviduct in a nine month old animal (scale bar = 100 μ m).

Between the epithelium and prostate gland there is a thick muscular layer which becomes thinner as the prostate grows. By eight months of age the female part has begun to fold and the two ducts are evident. The epithelium of the female duct is thick, while the male duct is composed of simple, cuboidal epithelium. The female glands are beginning to differentiate. By nine months the female portion is more folded, and the epithelium becomes thinner (Fig. 10). The cell glands are still small. In the male side, the prostate gland is bigger but no secretion is present. In some portions of the sperm duct it is possible to see the cells that will form the other two male glands, but they are not yet clearly differentiated. At ten months of age, the cells of the prostate begin to secrete eosinophilic globules. The other two glands are already differentiated. The female part is folded but only the cell glands close to the albumen gland are full of secretions.

At 12 months of age, female and male parts are well developed, the folds are deeper, and the epithelium of the female duct is composed of cuboidal cells with short cilia. All glands have increased in size, and those of the male portion have secretions in their cytoplasm. The epithelium of the male duct is columnar and has long cilia. By 14 months all female and male glands are full of secretions and are discharging their products into the ducts. The follicles of the prostate have increased in size so much that there is no space between them. All glands reach their maximum size by this time.

TERMINAL GENITALIA

Here are included the free oviduct, vas deferens, vagina, penis and bursa copulatrix. All these organs are simple in structure with an epithelium surrounded by muscular layers. When the walls begin to differentiate and fibers form, the epithelium commences to fold. All organs then enlarge until at the spermatozoa stage they all reach their definitive size.

Separation of the spermoviduct into two free ducts, the

vas deferens and the oviduct, occurs before four months old.

DISCUSSION

Maturity is defined here as the moment when sperm have been discharged into the hermaphroditic duct. All individuals that had reached their definitive shell form, with a reflected outer lip and a shell diameter of at least 30 mm, were mature. This stage is reached for most animals between ten and 12 months of age, when the ovotestis is already at the spermatozoa stage.

However, some individuals did not attain maturity until at least 12 months of age, when only spermatids, but not ripe sperm, were found in the ovotestis. Although these animals were from the same clutch of eggs and were kept under the same conditions, they developed at different rates. Usually they were smaller in size and some of them had some kind of shell damage. There is a relationship between shell diameter and degree of maturity reached by the reproductive tract, although this is not the only factor that affects development. I suggest that snails put their energy into building their shell and if something happens that damages the shell, development of the reproductive tract is stopped or slowed down, and the energy is used in repairing the shell only. This could explain the observation that all animals with damaged shells had immature reproductive tracts.

In *Neohelix major* there is a relationship between ovotestis stage and stage of maturation of the remainder of the tract. The "spermatocyte stage" means the beginning of the differentiation of the tract. At the "spermatid stage" all organs have grown and started to mature, and the "spermatozoa stage" marks the maturity of the female and male portions of the tract.

Several authors such as Laviolette (1954) and Runham *et al.* (1973), have demonstrated through different experiments on castration and transplantation of gonads or reproductive tracts, that the ovotestis influences the differentiation and functioning of the remainder of the tract. However, these kinds of experiments, such as castration, are difficult to carry out in shell-bearing snails in which the gonad is embedded in the digestive gland.

According to Boer and Joosse (1975), growth and differentiation of the reproductive tract of pulmonate gastropods is under the control of two endocrine factors produced by the gonad, one for the male and one for the female part. The production of each of these factors is controlled by hormones from the central nervous system. The male system completes maturation slightly earlier than the female system, although both male and female systems appear to be functional in the mature animal.

It would be necessary to compare this information from laboratory-reared snails with wild snails of the same species to check if maturation of the reproductive system is reached within the first year of life and if there is greater separation in time of the male and female functions. It seems that animals hatched and reared in the laboratory tend to mature more quickly than wild snails, probably because they have consistent "good" conditions of life, and hibernation or aestiva-

tion periods are shorter than in the field.

ACKNOWLEDGMENTS

My sincere thanks are due to Dr. William H. Heard of Florida State University for his advice and encouragement throughout this work and for the provision of laboratory facilities. I am indebted to Dr. Virginia Vail for providing her collection of laboratory reared snails and her helpful comments. For much help and encouragement I wish to thank Dr. Bowie Kotrla. I also thank Dr. Melbourne Carriker for critically reviewing the manuscript. I am pleased to thank the American Malacological Union for the special student award given for the presentation of this paper.

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Date of manuscript acceptance: 18 March 1990.

**SYMPOSIUM ON THE BIOLOGY
OF PELAGIC GASTROPODS**

ORGANIZED BY
ROGER SEAPY
CALIFORNIA STATE UNIVERSITY,
FULLERTON

AMERICAN MALACOLOGICAL UNION
LOS ANGELES, CALIFORNIA
26 JUNE 1989

MORPHOLOGY OF THE WINGS OF EUTHECOSOMATOUS PTEROPODS

DIETER FIEGE

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ABSTRACT

Using transmission and scanning electron microscopy, morphology and ultrastructure of the wings and the median footlobe of different euthecosomatous pteropods were investigated [*Limacina inflata* (Orbigny), *Creseis acicula* (Rang), *Clio pyramidata* (Linné), and *Cavolinia inflexa* (Lesueur)]. The cavity of the wings is filled with hemolymph-fluid. Dorsal and ventral wing epithelia are connected to each other by smooth muscle cells situated perpendicular to the wing plane. The cells are cross-linked and form a regular framework. In *Cavolinia inflexa*, these muscle cells form a septum in the median wing plane, parallel to the wing surface. This septum is in some parts perforated and acts as support for muscle fibres and blood vessels. A quite similar framework can also be found in the median footlobe of *Clio pyramidata* and *Cavolinia inflexa* with the exception of a median septum. This provides further evidence for a common origin of foot and wings.

In the veliger of *Creseis acicula*, the wings and velum, the larval swimming organ, are present at the same time in the same specimen. The velum, a transitional larval organ, disappears during metamorphosis (Fol, 1875). The wings develop as derivatives of the foot to the swimming organs of the adult.

Thecosomatous pteropods suffer two main disadvantages in their pelagic mode of life. Their bodies are far from being streamlined relative to their swimming direction (Schiemenz, 1895). Secondly, they bear a relatively heavy shell, which in the euthecosomatous pteropods is a true calcified shell in contrast to the gelatinous pseudoconch of many pseudothecosomatous species (Richter, 1973). The formation of a powerful swimming organ is therefore the most important adaptation to holoplanktonic life. In the euthecosomatous pteropods this is found as two lateral wings (= parapodia) which are derivatives of the gastropod foot. The animals swim by flapping their wings and therefore, commonly are called "sea butterflies".

This way of swimming suggests a complex muscle structure in the wings, which has been studied by several authors using light microscopy (LM) (Meisenheimer, 1905, v.d. Spoel, 1967; Pafort-v. Iersel and v.d. Spoel, 1979; Pafort-v. Iersel, 1979). So far there have been no investigations of the wings of euthecosomatous pteropods using electron microscopy.

MATERIALS AND METHODS

Juvenile (metamorphosed) specimens of *Limacina*

inflata (Orbigny), *Creseis acicula* (Rang), *Clio pyramidata* (Linné) and *Cavolinia inflexa* (Lesueur) (Figs. 1 a-d, 2a) were collected in plankton tows at Villefranche-sur-mer, France, during 1987 and 1988. Veliger larvae of *Creseis acicula* (Figs. 3a, b) were collected in Banyuls-sur-mer, France, in 1985. Specimens were relaxed using 7% MgCl₂ in distilled water, fixed in 2.5% glutaraldehyde in 0.1 mol/l Na-cacodylate buffer (adjusted to 900 mOsmol with NaCl, pH 7.2 - 7.4). Decalcification of the shells was performed by addition of ethylenediamine tetraacetate (EDTA) to the primary fixative (Bonar and Hadfield, 1974). The specimens were rinsed in 0.1 mol/l Na-cacodylate buffer (adjusted to 1200 mOsmol with NaCl, pH 7.2 - 7.4) and postfixed in 2% OsO₄ in the same buffer, dehydrated via graded alcohol series and embedded in Spurr's medium (Spurr, 1969). After sectioning up to the region of interest using a Reichert Ultracut, they were de-embedded (Hogan and Smith, 1982; modified after Russel and Daghljan, 1985), critical-point dried, coated with gold and examined with a Hitachi scanning electron microscope (SEM) H-530.

Ultrathin sections were stained with 1% lead citrate (Reynolds, 1963) and examined in a Philips transmission electron microscope (TEM) 201. Using both methods for the same specimen the results complement each other (Figs. 4a, b).

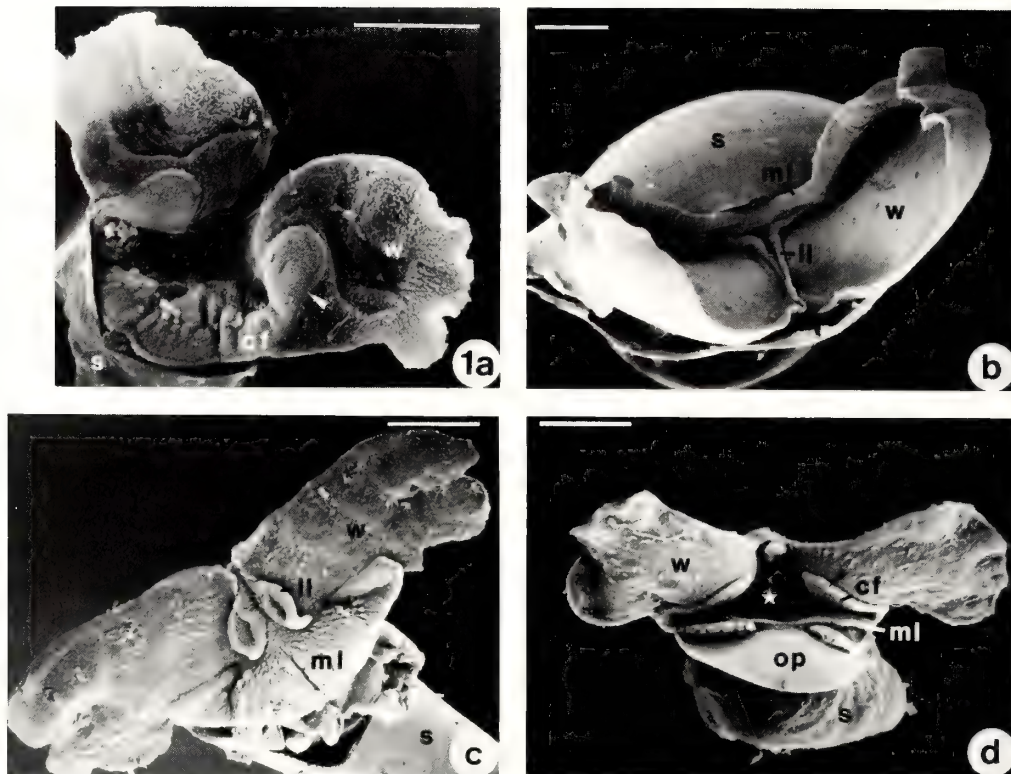


Fig. 1. Ventral view of examined specimens showing different parts and derivatives of the foot: **a**, *Creseis acicula*; **b**, *Cavolinia inflexa*; **c**, *Clio pyramidata*; **d**, *Limacina inflata* (cf, ciliated field; ml, median footlobe; ll, lateral footlobe; op, operculum; s, shell; w, wing; *, artefact; ►, bordering band of cilia) (scale bars: **a**, **d**: 200 μ m; **b**, **c**: 500 μ m).

RESULTS

I. EXTERNAL MORPHOLOGY OF THE WINGS AND FOOTLOBES

In euthecosomatous pteropods, the foot consists of several parts, one median (posterior) and two lateral lobes. Viewing the animal from the ventral side these footlobes can be recognized by their dense ciliation (Figs. 1b-d). The wings are situated dorsally from the footlobes. In some species, such as *Creseis acicula*, the cilia are not restricted to the footlobes but spread onto the ventral wing surface (Fig. 1a). In *Creseis acicula*, *Limacina inflata* and *Cavolinia inflexa* this ciliated field is bordered by a less dense band of differently orientated cilia (Figs. 1a, d, 4a, 5a). The cells bearing the ciliated field are much more voluminous than the flat, polygonal cells of the adjacent wing epithelium (Figs. 2b, 4a, b).

Some *Cavolinia inflexa* show a distinct row of cilia on the ventral surface of the left wing with the cilia arranged longitudinally in small, separate groups (Fig. 5b). In one specimen they are also present on the ventral surface of the right wing, but are less prominent. They are missing in the specimen shown in figure 1b.

II. INNER STRUCTURE OF WINGS AND MEDIAN FOOTLOBE

Two different muscle systems can be distinguished in

the wings: subepithelial musculature and a framework of muscle cells inside the wing cavity (Meisenheimer, 1905). The subepithelial musculature consists of bundles of transversely striated muscle fibres [Figs. 2b, subepithelial musculature (sm) seen through epithelium, 2c, 4a, also see Fig. 6f]. The nucleus and mitochondria are situated in the center of the fibre and surrounded by muscle filaments (Figs. 2c, 7a, b). In *Limacina inflata*, the subepithelial musculature forms only a single layer (Fig. 2b).

In the wing cavity, which is filled with hemolymph-fluid, muscle cells ("wing bars", Pafort-v. Iersel, 1979) are situated in regular intervals perpendicular to the wing plane forming a framework (*Creseis acicula*: Figs. 4b, 8a, b; *Clio pyramidata*: Figs. 8c, d). In contrast to the subepithelial muscles, they consist of smooth musculature (Figs. 7c, 9a, c). Their dorsal and ventral tips divide into branches (mostly two) before they penetrate the subepithelial muscle layers (Figs. 6e, 9c) and divide again before they insert at the epithelia with desmosomes (Figs. 9b, c). Their nuclei are situated in the median wing plane (Fig. 8a). From this level muscle fibres connect neighbouring cells, thus forming rows of "pylons" parallel to the long axis of the wing. These rows are often connected to each other by horizontal branches also originating from the nuclear region (Fig. 8a).

In *Cavolinia inflexa*, the muscle filaments perpendicular and parallel to the wing plane belong to the same cell as can

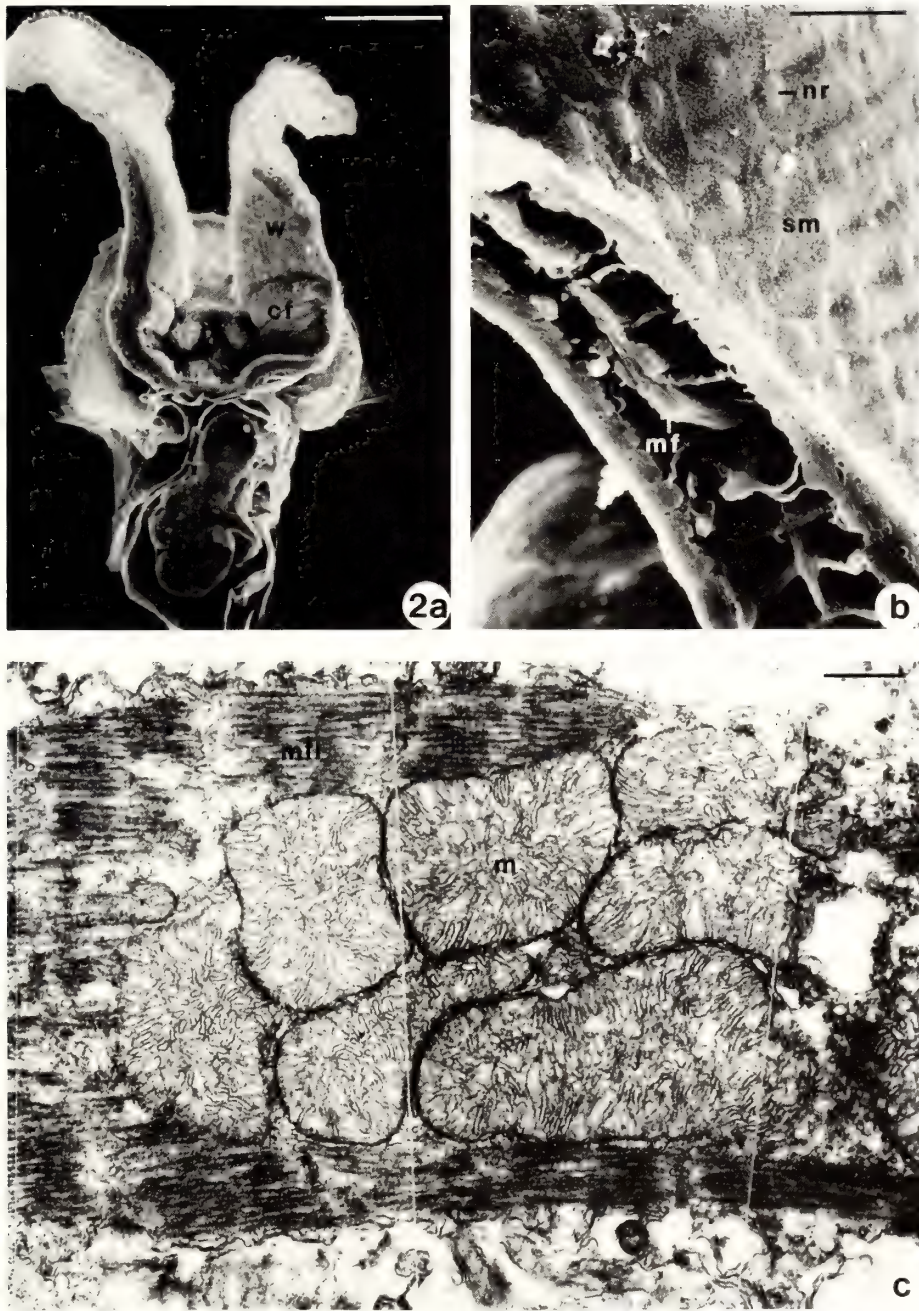


Fig. 2. Wing musculature of *Limacina inflata*: **a**, SEM micrograph of the whole animal showing sectioning plane; **b**, SEM micrograph of the wing showing wing musculature in section and underlying the wing epithelium, which is formed by flat, polygonal cells; **c**, TEM micrograph of a cross-section through subepithelial muscle fibre showing cell organelles (mitochondria) surrounded by muscle filaments (cf, ciliated field; m, mitochondrion; mf, muscle fibre; mfl, muscle filament; nr, nuclear region; sm, subepithelial musculature; w, wing) (scale bars: **a**, 0.5 μm ; **b**, 100 μm ; **c**, 20 μm).

be seen by the bending of the muscle filaments (Figs. 9a, c). These cells form a septum in the median wing plane, running parallel to the dorsal and ventral wing surface. The septum acts as support for muscle fibres originating from the columellar muscle and for blood vessels (Figs. 6a, b). The blood vessels are always situated on the ventral side of the septum

which is best developed in the largest part of the wing, but is often perforated (Fig. 6c) and diminishes towards the flattened front and edges of the wing. The muscular framework of the other species also becomes less regular towards the edges of the wing (Fig. 8c, *Clio pyramidata*).

In the wing lumen of juvenile *Clio pyramidata*, the same

framework of supporting structures can be found, with the exception of a median septum. Prolongations of the columellar muscle and blood vessels are loosely attached to cross-links in the median wing plane (Fig. 8d). The framework of supporting structures extends into the median footlobe, but a median septum is missing in this footpart in *Cavolinia inflexa* and *Clio pyramidata* (Figs. 5a-d). The more voluminous median footlobe of *Clio pyramidata* contains a much looser framework of muscle cells with few median cross-links (Figs. 5c, d).

III. ORIGIN OF THE WINGS

In the veliger of *Creseis acicula*, two lateral protrusions

of the foot can be observed in addition to typical larval features such as a well developed velum and a median band of cilia on the foot (Figs. 3a, b). In the specimen shown in figure 3b, the lateral protrusions are somewhat shorter than those of the one shown in figure 3a, indicating that this specimen probably is younger. Its lobes are round in cross-section and their inner structure is not yet differentiated. Only an inner and an outer layer of cells can be distinguished (Fig. 3c). The surface of the protrusions is covered with microvilli connected to each other by a "basket-work border" (Thompson, 1967). Later the lobes become flattened and develop an edge (Fig. 3a).

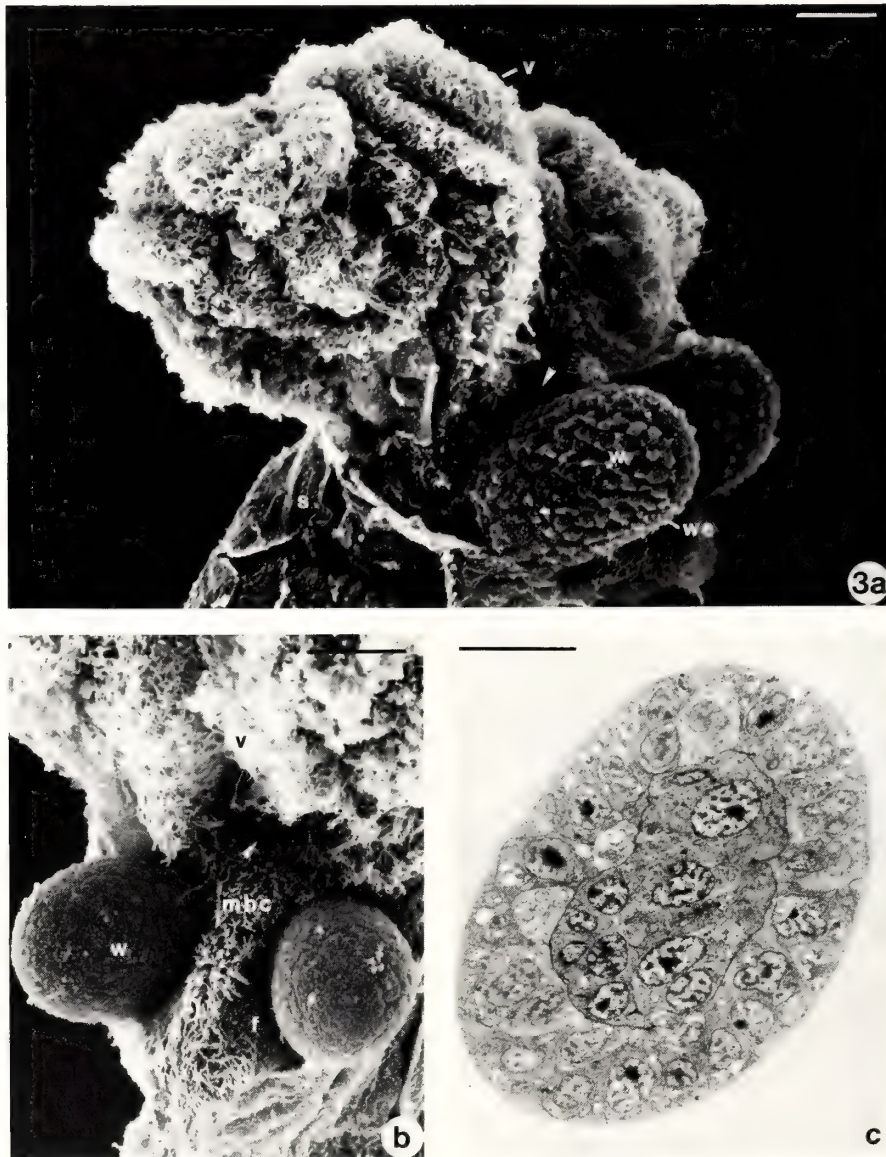


Fig. 3. Veliger of *Creseis acicula*: **a**, Veliger with wings and velum extended from the shell; **b**, Ventral view of a veliger showing velum and wings inserting on both sides of the foot, which bears a median band of cilia; **c**, TEM micrograph of a cross section through the wing in early larval stage (f, foot; mbc, median band of cilia; s, shell; v, velum; w, wing; we, wing edge; ►, position of the mouth) (scale bars: **a**, **b**, 20 μm ; **c**, 10 μm).

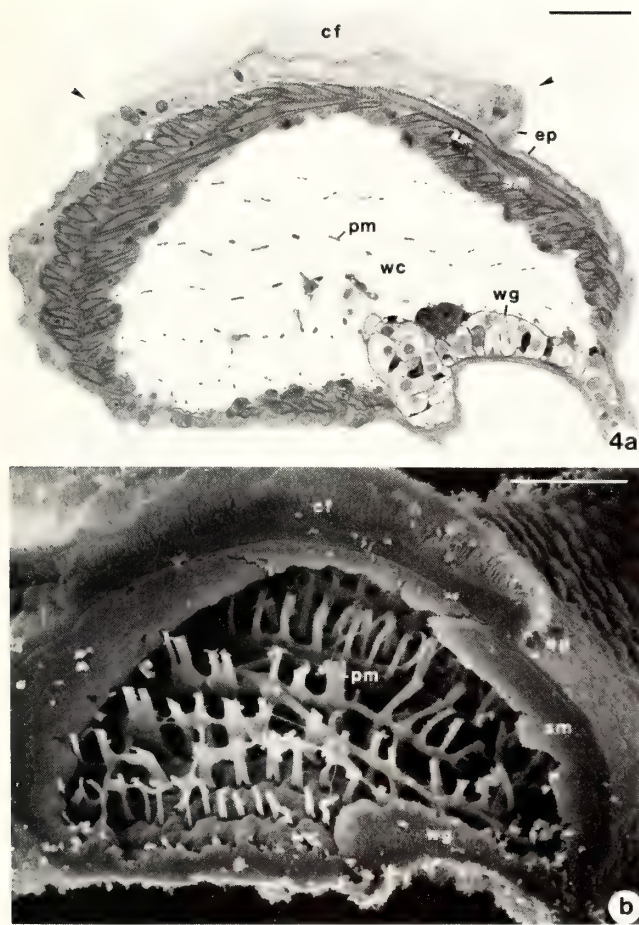


Fig. 4. Sagittal section through the wing of *Creseis acicula* showing muscle framework (a and b from the same specimen): a, Semi-thin section corresponding to b; b, SEM micrograph of the wing after sectioning (cf, ciliated field; ep, epithelium; pm, perpendicular muscle cell; sm, subepithelial musculature; wc, wing cavity; wg, "wing gland"; ►, bordering band of cilia) (scale bars: 40 μ m).

DISCUSSION

I. ROLE OF CILIA IN FEEDING

The differences in the external morphology of the distinct parts of the foot can be related to functional differences. According to Meisenheimer (1905) and Yonge (1926) the ciliated parts of the foot are used in funneling food particles towards the mouth, whereas the wings are used for locomotion.

Recent investigations of Gilmer and Harbison (1986) show that the animals use an external mucous web for feeding. However, it is not yet certain whether thecosomes are trappers (Richter, 1977) or suspension feeders.

The less dense cilia of the band surrounding the ciliated field in *Limacina inflata*, *Creseis acicula* and *Cavolinia inflexa* could be responsible for keeping food particles on the ciliated field. The effect could be caused by a different direction of the ciliary beat due to their different orientation.

So far there is not enough information to explain why the row of cilia on the ventral surface of the left wing of *Cavolinia inflexa* is present in one specimen and missing in the other. Observations of living specimens are highly desirable to study the function of the cilia row. So far it can only be speculated whether it takes part in the formation or retraction of the mucous web. A possible sensory function of these cilia is subject of current work.

II. ROLE OF THE SMOOTH MUSCULATURE IN LOCOMOTION

Two independent muscle systems can be found inside the wings and median footlobe: subepithelial musculature of transversely striated type and a central muscle framework (Meisenheimer, 1905). The smooth musculature composing the framework inside the cavity could be regarded as a derivative of the columellar muscle, taking part in the retraction of the wings and other footparts into the protecting shell (v. d. Spoel, 1967).

A contraction of these perpendicular muscle cells would also result in flattening of the wings in dorso-ventral direction thus altering the wing profile. This would lead to changes in its hydrodynamics and enable the animal to steer while swimming. In addition it could be responsible for maintaining the structure of the expanded wings while the subepithelial muscle layers do the flapping motion. This hypothesis is strengthened by the fact that the framework consists of smooth musculature. It is able to keep up a certain tension for a long period of time, whereas transversely striated musculature is known for strong but short movements. The pressure of the hemolymph-fluid alone might not be able to act as an appropriate antagonist to the strong movements of the striated musculature. A relatively high pressure would be necessary to maintain the wings shape and it seems doubtful whether the diaphragm of connective tissue (Meisenheimer, 1905), which divides the body cavity from the cavity of the wings and footparts, could withstand it.

III. ROLE OF THE SMOOTH MUSCULATURE IN CIRCULATING HEMOLYMPH-FLUID

The framework of smooth muscle cells could also take part in the circulation of the hemolymph-fluid. Thecosomes possess a partly closed circulatory system. Vessels running from the heart to the wings and to some inner organs can be found and also short vessels leading from the body cavity back to the heart (Meisenheimer, 1905). The hemolymph-fluid enters the wing via blood vessels belonging to the artery system. With the muscle framework performing rhythmical contractions resulting in a pumping mechanism the hemolymph-fluid might be pumped back into the body cavity passing valves in the diaphragm, which are known for *Cavolinia* (Meisenheimer, 1905).

As the blood vessels are always situated on the ventral side of the median septum, a directed flow of the hemolymph-fluid from the ventral part of the wings hemocoelic space along the septum and through its pores to the dorsal space and back into the body cavity could result. If this is

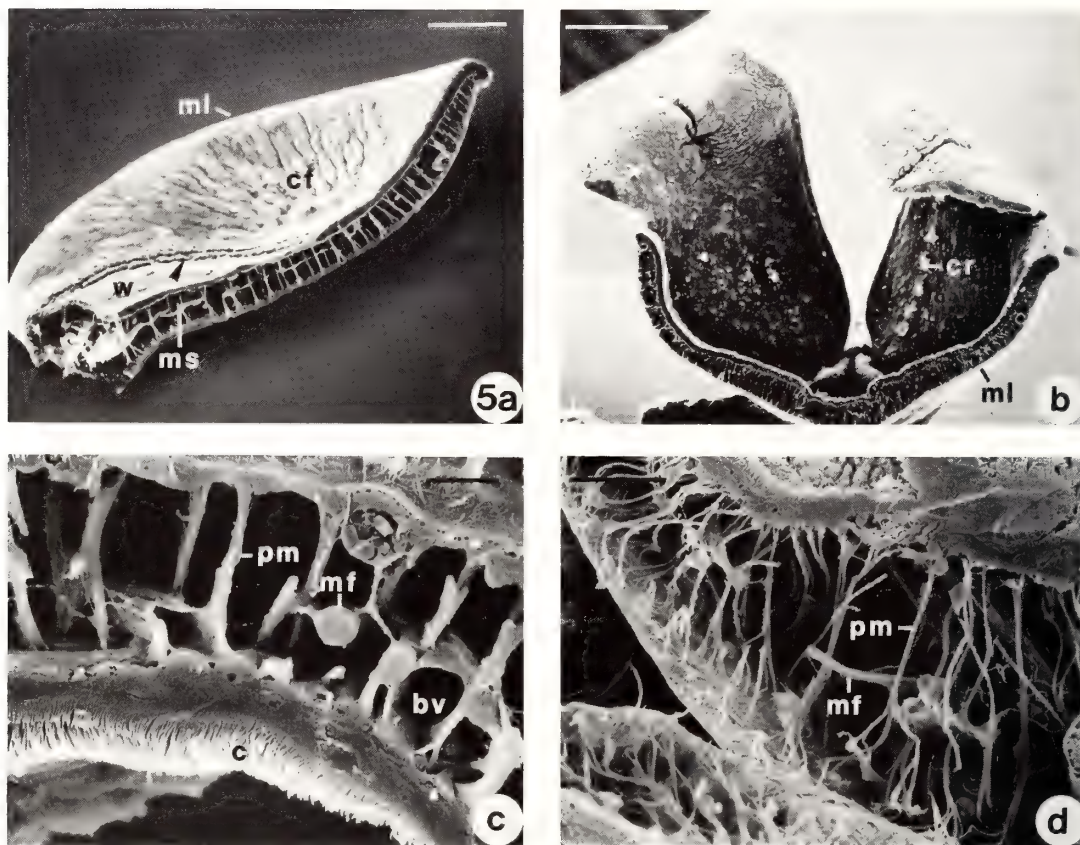


Fig. 5. SEM micrographs of the musculature of the median footlobe of *Cavolina inflexa* (a, b) and *Clio pyramidata* (c, d): a, Part of the median footlobe with its ciliated field, the bordering band of cilia and the adjacent wing. The median septum is present in the cavity of the wing but missing in the median footlobe; b, SEM micrograph showing sectioning plane through median footlobe. An interrupted line of cilia can be seen on the ventral surface of the left wing; c, Detail of the median footlobe. Cross-links between the perpendicular muscle cells are present but no median septum; d, Section through the proximal part of the median footlobe showing loose and less regular framework of perpendicular muscle cells. A median septum is missing (bv, blood vessel; c, cilia; cf, ciliated field; cr, cilia row; mf, muscle fibre; ml, median footlobe; ms, median septum; pm, perpendicular muscle cell; w, wing; ►, bordering band of cilia) (scale bars: a, 100 μ m; b, 200 μ m; c, 10 μ m; d, 40 μ m).

true, the wings, with their enormous surface area could play a major role in gas exchange, especially in species without secondary gills. Perpendicular structures similar to those in the wings are also found in the mantle appendages of *Cavolina inflexa*. They have not yet been shown to be smooth muscle cells, but the mantle appendages could also take part in gas exchange.

IV. STRUCTURE OF WINGS AND MEDIAN FOOTLOBE

The fact that the same supporting structures are found in the cavities of the wings and median footlobe provides evidence for a common origin of foot and wings in euthecosomatous pteropods, as described by Fol (1875). However, there is no median septum in the median footlobe perhaps because it is relatively small and does not take part in the swimming motion, which makes additional stiffening superfluous. A similar arrangement of supporting structures can also be found in the gymnosome *Clione limacina* (Satterlie *et al.*, 1985) and in the pseudothecosomes *Peraclis*

reticulata (Orbigny) (Fiege, unpub. data) and *Cymbulia peronii* (Blainville) (Pafort-v. Iersel, 1979).

V. ORIGIN OF THE WINGS

The development of pteropods was first described by Fol (1875). According to his findings the wings and the foot have a common origin, i.e. the wings are derivatives of the foot. Van der Spoel (1982), however, suggests that wings and velum of thecosomatous pteropods are homologous organs relying on the wings apically and dorsally position to the mouth (Pafort-v. Iersel and v. d. Spoel, 1979, and quoted in v. d. Spoel, 1982).

The results presented here for veliger larvae of *Creseis acicula* support Fol's observations. In the larva two lateral protrusions of the foot can be seen, and, at the same time, typical larval features like the median band of cilia on the foot and a well developed velum (Figs. 3a, b). The velum, a transitional larval organ in adaptation to planktonic development, disappears during metamorphosis [Fol, 1875; Lebour, 1932 for *Limacina retroversa* (Fleming)]. So intermediate forms between

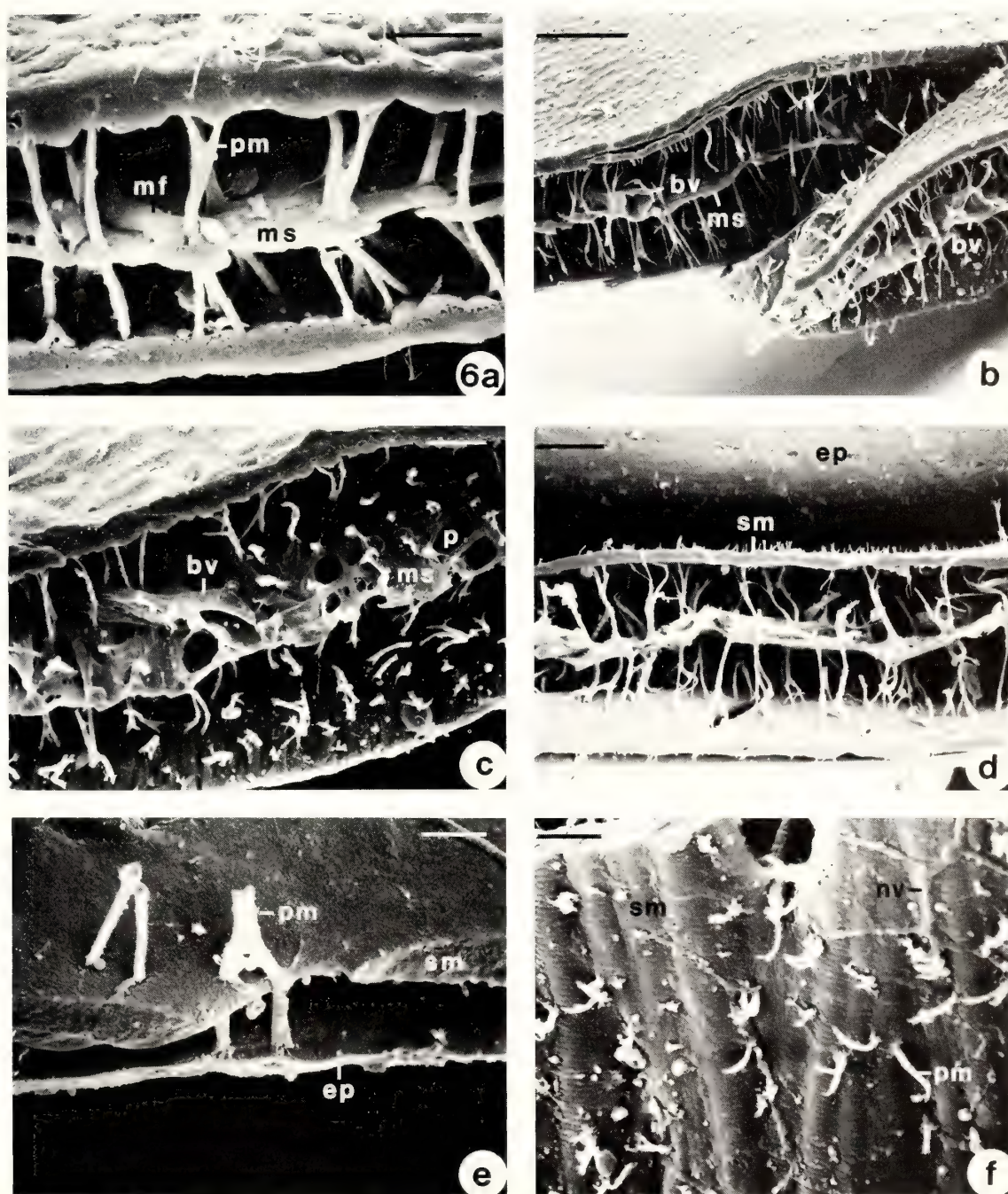


Fig. 6. SEM micrographs of the muscle framework inside the wings of *Cavolinia inflexa*. Transverse sections through the wing showing: **a**, A well developed median septum supporting a muscle fibre; **b**, A blood vessel attached to the median septum; **c**, Pores in the median septum; **d - f**, SEM micrographs taken from the same specimen. The subepithelial musculature was torn off from the epithelium during preparation: **d**, Subepithelial musculature torn off from the epithelium; **e**, Smooth, perpendicular muscle cells penetrating subepithelial muscle layer inserting at the epithelium; **f**, Outer side of the subepithelial muscle layer showing banding pattern of transversely striated musculature, nerves and penetrating branches of perpendicular muscle cells (bv, blood vessel; ep, epithelium; mf, muscle fibre; ms, median septum; nv, nerve; p, porus; pm, perpendicular muscle cell; sm, subepithelial musculature) (scale bars: **a**, **c**, 20 μm ; **b**, 100 μm ; **d**, 40 μm ; **e**, **f**, 10 μm).

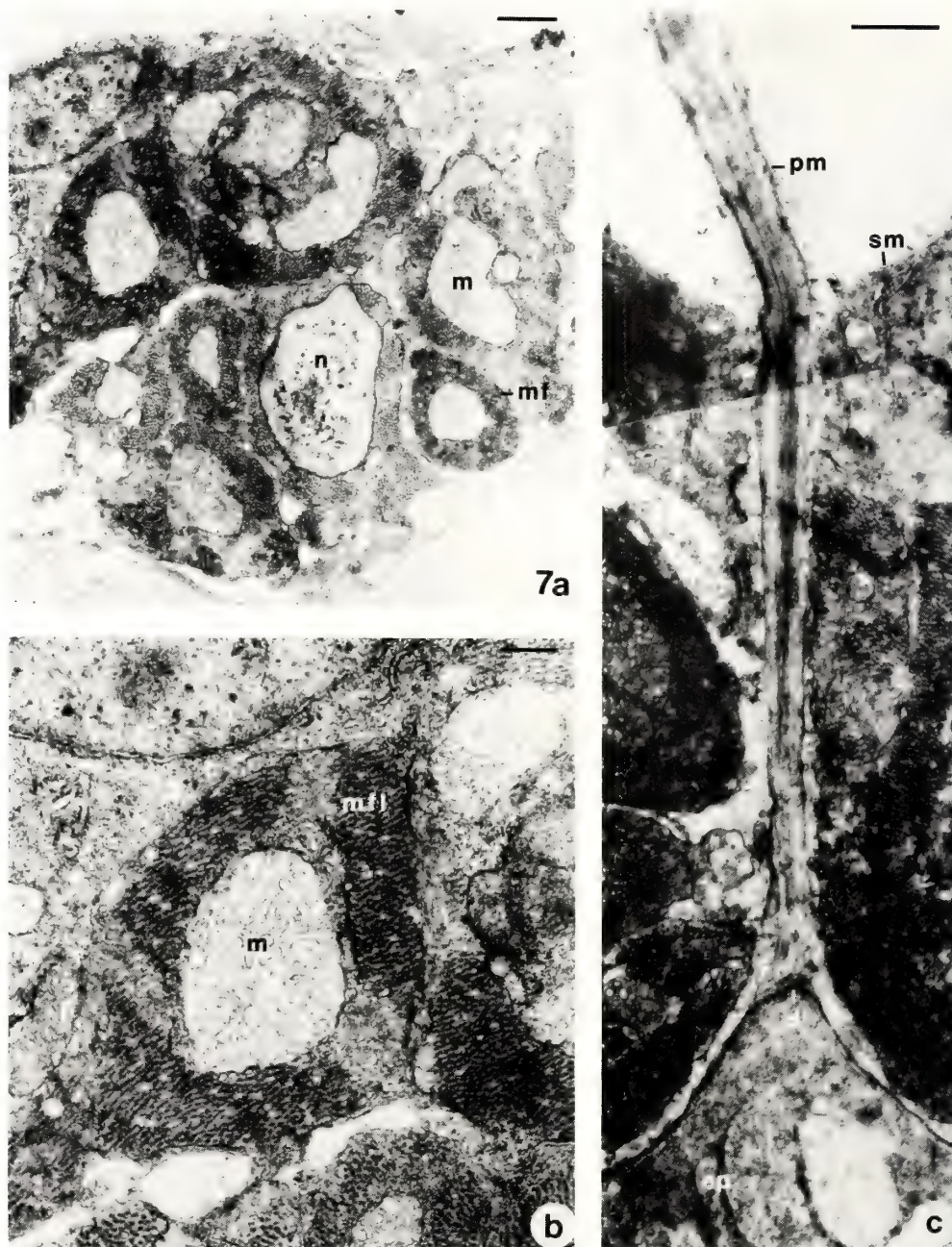


Fig. 7. TEM micrographs of the wing musculature of *Creseis acicula*: **a**, Cross-section through a bundle of muscle fibres in subepithelial musculature showing cell organelles surrounded by muscle filaments; **b**, Detailed view of a single muscle fibre; **c**, Perpendicular muscle cell running through subepithelial musculature inserting at the epithelium (d, desmosome; ep, epithelium; m, mitochondrion; mf, muscle fibre; mfl, muscle filament; pm, perpendicular muscle cell; n, nucleus; sm, subepithelial musculature) (scale bars: 1 μ m).

velum and wings are not very likely and have not been described by Fol (1875) for the ontogeny of the pteropods.

The wings of the adult substitute the larval velum in its main function, locomotion of the animal. However this is done in a completely different way according to the heteromorphic structure of the organs. In contrast to metamorphosed pteropods, in which locomotion is provided by flapping of the

wings (Morton, 1964), locomotion in veliger larvae is provided by the beat of cilia located on the edge of the velum (Fretter, 1967).

As both organs are present at the same time in different positions, they can not be regarded as homotopic. In the veliger the velum is found apically and dorsally and the wings first appear caudally in relation to the mouth on both sides

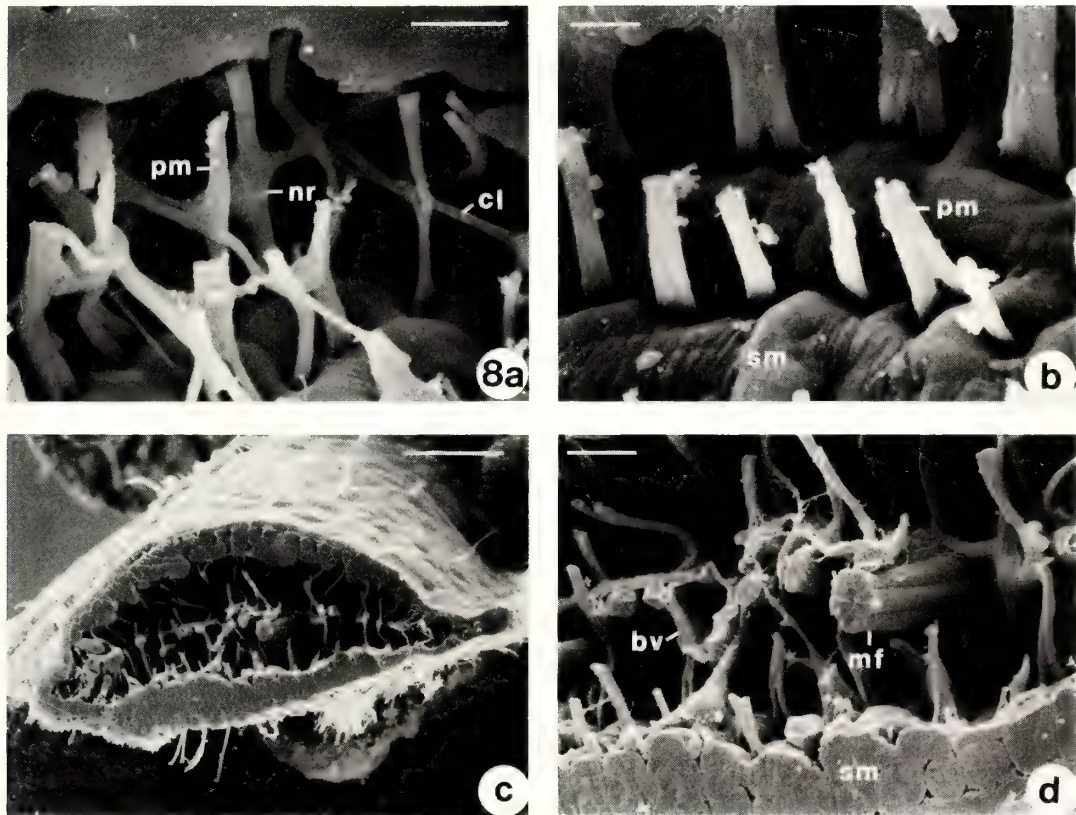


Fig. 8. SEM micrographs of the muscle framework inside the wings of *Creseis acicula* (a, b) and *Clio pyramidata* (c, d): a, Cross-links and nuclear regions of perpendicular smooth muscle cells; b, Perpendicular muscle cells and transversely striated subepithelial musculature showing regular banding pattern; c, Cross section through the right wing. No median septum is present; d, Detail of c showing muscle fibres and blood vessels attached to cross-links of perpendicular muscle cells (bv, blood vessel; cl, cross-link; mf, muscle fibre; nr, nuclear region; pm, perpendicular muscle cell; sm, subepithelial musculature) (scale bars: a, d, 10 μ m; b, 5 μ m; c, 40 μ m).

of the foot. After metamorphosis the wings take a position dorso-apically to the mouth. Thus the wings substitute the velum not only in its function but also in its position. Therefore they were probably considered to be homologous by v. d. Spoel (1982). However, based on the results presented in this study, the wings and velum can not be regarded as homologous organs.

ACKNOWLEDGMENTS

It is a pleasure to thank Prof. Dr. P. Fioroni for bringing pelagic gastropods to my attention, G. Wildenburg and M. Meschenmoser for critical comments on the manuscript, Dr. G. Richter for his help in determination of the species, and Dr. S. Nival, Director of the Station Zoologique de Villefranche-sur-mer, and Dr. J. Soyer, Director of the Laboratoire Arago in Banyuls-sur-mer, for providing research facilities.

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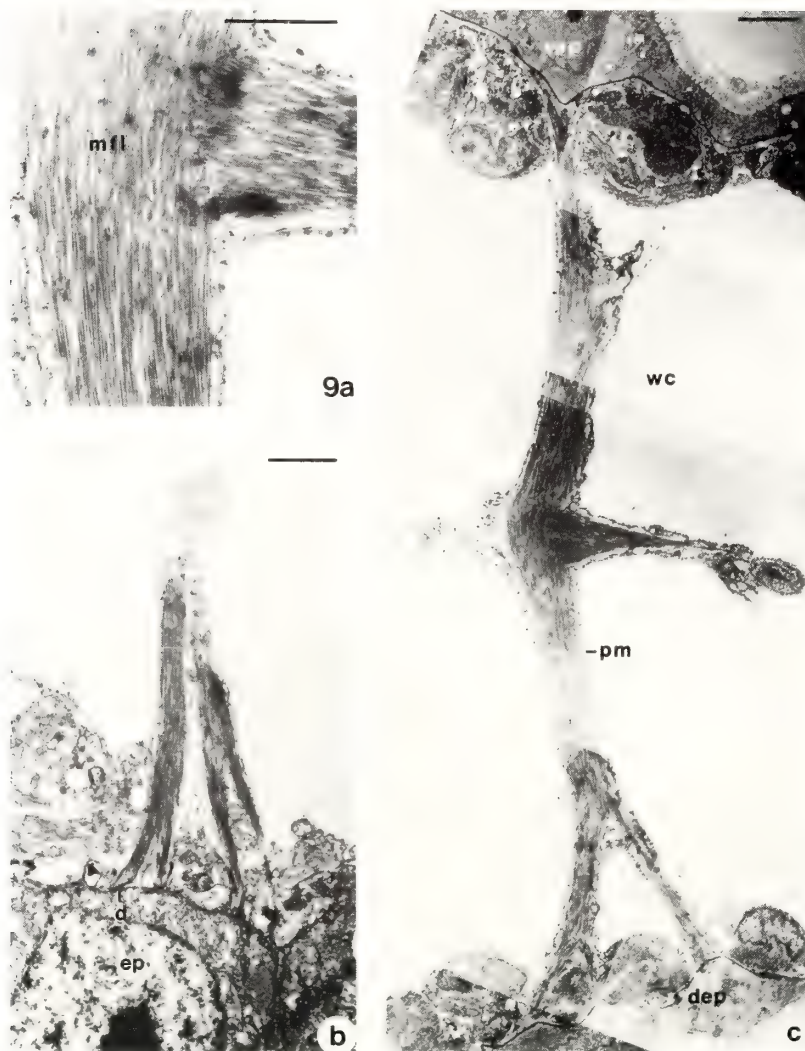


Fig. 9. TEM micrographs of the perpendicular muscle cells in the wing cavity of *Cavolinia inflexa* showing branching and insertion at the epithelium: **a**, Detail of **c** showing muscle filaments bending from perpendicular to horizontal direction forming cross-links between neighbouring muscle cells; **b**, Branching of a perpendicular muscle cell before inserting at the epithelium with desmosomes; **c**, Perpendicular muscle cell connecting dorsal and ventral wing epithelium (d, desmosome; dep, dorsal epithelium; ep, epithelium; mfl, muscle filament; pm, perpendicular muscle cell; vep, ventral epithelium; wc, wing cavity) (scale bars: **a**, **b**, 2 μ m; **c**, 4 μ m).

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Date of manuscript acceptance: 27 September 1989

KARYOTYPE ANALYSIS IN SEVERAL PELAGIC GASTROPODS

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ABSTRACT

Chromosome number and morphology were determined for six species of pelagic gastropods: the Thecosomata *Hyalocylis striata* Rang ($2n=28$); the Gymnosomata *Pneumodermopsis paucidens* Boas ($2n=32$) and *Paraclione longicaudata* Souleyet ($2n=32$); the Heteropoda *Pterotrachea coronata* Forskal (female $2n=32$, male $2n=31$), *Pterotrachea hippocampus* Philippi (male $2n=31$) and *Firoloida desmaresti* Lesueur (female $2n=32$, male $2n=31$). The three latter species displayed male karyotypes with heteromorphic chromosomes which suggest a XY_1Y_2 sex mechanism. Comparison of known karyological data among the Thecosomata shows an evolutionary trend from low diploid complement with metacentric chromosomes [as in *Limacina inflata* (Orbigny)] toward higher diploid complement and an increase in the proportion of subtelocentric-telocentric chromosomes (as in *Cymbulia peroni* De Blainville). Within the Gymnosomata, the known four species reveal a chromosomal stability in the chromosome number and a variable proportion of metacentric-submetacentric and subtelocentric-telocentric chromosomes. The chromosome diploid number in Heteropoda ranges close to the diploid complement found in Hydrobiidae, Littorinidae and Naticidae. The unusual sex-determining mechanism XY_1Y_2 is discussed among the Mesogastropoda.

Chromosomal data on pelagic gastropods have been limited to the knowledge of the chromosome number for five species of pteropods (Nakrassov, 1904; Zarnick, 1911) and two species of heteropods (Boveri, 1890). The development of cytogenetics has yielded karyological investigations in many molluscan species (e.g. see reviews by Nakamura, 1985, 1986), but none on pelagic gastropods. Yet, the holoplanktonic animals are of particular interest among gastropods in illustrating the evolution of unusual adaptation to the pelagic environment. Furthermore, three taxonomical groups of pelagic gastropods, i.e. two opisthobranch orders, Thecosomata and Gymnosomata, and one prosobranch group, Heteropoda, show important anatomical variations within their groups. The phylogenetic origin of these taxons remains obscure and have led to considerable speculation (Lalli and Gilmer, 1989). Cytogenetic parameters such as chromosome number and morphology can also be used as an investigatory tool to trace phylogenetic patterns and evolutionary relationships. With this aim in mind, chromosome studies on seven species of Thecosomata and two species of Gymnosomata were first carried out (Thiriot-Quévrex, 1988). In the present paper, chromosome investigations are added for one species of Thecosomata, two species of Gymnosomata, and three species of Heteropoda. Evolutionary cytotaxonomy and karyological tendencies of these taxons are discussed.

MATERIALS AND METHODS

Pelagic gastropods examined in this paper were collected from plankton tows taken at 20 m in the bay of Villefranche-sur-Mer (Mediterranean coast of France). The following species were investigated: Thecosomata Euthecosomata Cavoliniidae: *Hyalocylis striata* Rang; Gymnosomata Pneumodermatiidae: *Pneumodermopsis paucidens* Boas; Gymnosomata Clionidae: *Paraclione longicaudata* Souleyet; Heteropoda Pterotracheidae: *Pterotrachea coronata* Forskal, *Pterotrachea hippocampus* Philippi and *Firoloida desmaresti* Lesueur.

Chromosome preparations were from gonads during gametogenesis according to techniques described by Thiriot-Quévrex (1988). Staining was effected with Giemsa 4%, pH 6.8 and according to Howell and Black (1980) for NORs (nucleolar organizer region). Chromosome measurements and statistical interpretations were made with a CHROMOS program (Thiriot-Quévrex, 1984; Thiriot-Quévrex *et al.*, 1988).

Relative length (= percent total complement length) was expressed as the ratio of absolute chromosome pair length over total length of haploid complement $\times 100$. Centromeric index was calculated as the ratio of length of short arm over total length of chromosomes $\times 100$. Arm ratio was calculated as length of short arm/length of long arm.

Terminology relating to centromere position follows that of Levan *et al.* (1964).

RESULTS

THECOSOMATA: *HYALOCYLIS STRIATA*

At mitotic metaphases, the diploid number of chromosomes is $2n=28$. Figure 1 shows the karyotype arranged by decreasing chromosome size. Table 1 gives the measurements and classification of four selected metaphase plates. Pairs 1, 2, 3, 4, 9, 11 and 13 are metacentric, pairs 12 and 14 are submetacentric, pair 10 is subtelocentric-submetacentric (the centromere position being on the borderline between two different categories with 95% confidence limits), pairs 5, 6, 7 and 8 are subtelocentric. The ideogram (Fig. 2) constructed from relative length and centromeric index values visualizes the karyotype with the different morphological types of chromosomes.

GYMNOSOMATA: *PNEUMODERMOPSIS PAUCIDENS*

Mitotic metaphases were observed with a diploid set of $2n=32$. Eight selected spreads from different animals were karyotyped and measured (Table 2). In the karyotype (Fig. 3A),

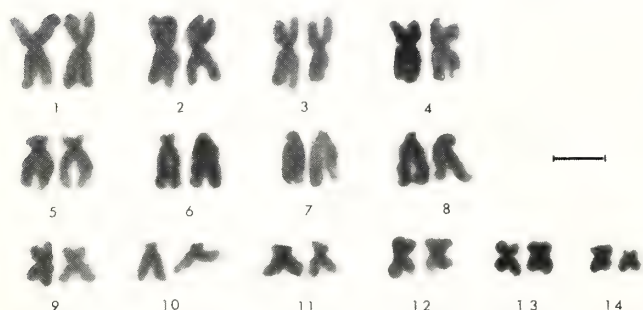


Fig. 1. Karyotype of *Hyalocylis striata* (scale bar = 5 μ m).

the first metacentric pair is about five times longer than the last pair and conspicuously larger than the remaining pairs whose size decrease progressively. Pairs 1, 2, 3, 4, 6, 12 and 14 are metacentric, pairs 8, 9, 10, 13, 15 and 16 are submetacentric, pair 11 is subtelocentric-submetacentric, and pair 7 telocentric.

Comparison of the same metaphase stained with standard Giemsa (Fig. 4C) then silver-stained (Fig. 4D) showed that the NORs are located on the metacentric pair 2. Silver-stained metaphases in *Pneumodermopsis canephora* Pruvot-Fol (Fig. 4A) and *Pneumoderma atlanticum* Oken (Fig. 4B) showed NORs on the short arm of the subtelocentric pair 2.

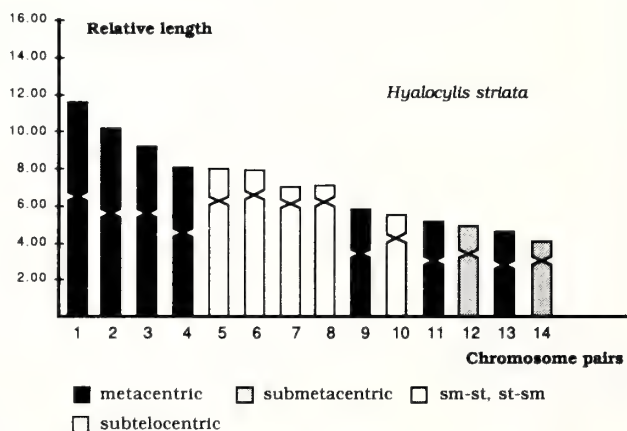


Fig. 2. Ideogram of *Hyalocylis striata* constructed from relative length and centromeric index values. Chromosome morphology is indicated: dark stippled for metacentric, medium stippled for submetacentric, light stippled for subtelocentric-submetacentric or subtelocentric-submetacentric (i.e. $23.06 < Ci < 26.74$), open for subtelocentric.

Table 1. Chromosome measurements and classification in four metaphasic cells of *Hyalocylis striata*.

Chromosome pair no.	Relative length		Arm ratio		Centromeric index		Classification*
	Mean	SD	Mean	SD	Mean	SD	
1	11.61	0.813	0.796	0.037	44.15	1.232	m
2	10.30	0.339	0.848	0.151	44.84	3.840	m
3	9.29	0.366	0.675	0.087	39.51	3.175	m
4	8.02	0.455	0.834	0.146	44.82	4.707	m
5	8.07	0.434	0.297	0.090	22.40	5.616	st
6	7.92	0.437	0.213	0.085	17.03	5.487	st
7	7.12	0.442	0.149	0.020	12.87	1.556	st-t
8	7.01	0.367	0.150	0.055	12.78	4.132	st-t
9	5.97	0.165	0.724	0.131	41.23	4.452	m
10	5.49	0.149	0.311	0.089	23.30	5.114	st-sm
11	5.37	0.214	0.727	0.269	40.04	7.063	m
12	4.97	0.162	0.491	0.372	29.14	17.240	sm
13	4.70	0.425	0.785	0.222	41.77	4.586	m
14	4.11	0.364	0.381	0.052	27.15	2.712	sm

*m = metacentric; st = subtelocentric; st-t = subtelocentric-telocentric; st-sm = subtelocentric-submetacentric; sm = submetacentric.

Table 2. Chromosome measurements and classification in eight metaphasic cells of *Pneumodermopsis paucidens*.

Chromosome pair no.	Relative length		Arm ratio		Centromeric index		Classification*
	Mean	SD	Mean	SD	Mean	SD	
1	13.48	0.628	0.880	0.056	46.60	1.580	m
2	8.83	0.561	0.701	0.599	40.63	2.595	m
3	8.15	0.328	0.809	0.109	44.08	3.320	m
4	7.96	0.371	0.698	0.162	40.18	5.822	m
5	7.22	0.501	0.319	0.095	23.42	5.173	st
6	7.11	0.244	0.716	0.167	40.58	5.290	m
7	6.22	0.667	0.132	0.044	11.39	3.277	t
8	6.07	0.464	0.403	0.126	27.82	6.678	sm
9	6.06	0.479	0.633	0.264	36.50	8.493	sm-m
10	5.46	0.368	0.545	0.144	34.47	6.854	sm
11	5.37	0.510	0.322	0.204	22.62	10.820	st-sm
12	4.55	0.238	0.700	0.161	39.95	5.713	m
13	4.22	0.271	0.441	0.148	29.34	5.995	sm
14	3.90	0.239	0.692	0.129	39.93	3.940	m
15	2.90	0.252	0.420	0.079	28.84	4.012	sm
16	2.41	0.341	0.543	0.173	33.93	7.036	sm

*m = metacentric; st = subtelocentric; t = telocentric; sm = submetacentric; sm-m = submetacentric-metacentric; st-sm = subtelocentric-submetacentric.

GYMNOSOMATA: *PARACLIONE LONGICAUDATA*

Thirty-two chromosomes were counted in mitotic metaphases. Table 3 gives chromosome measurements on

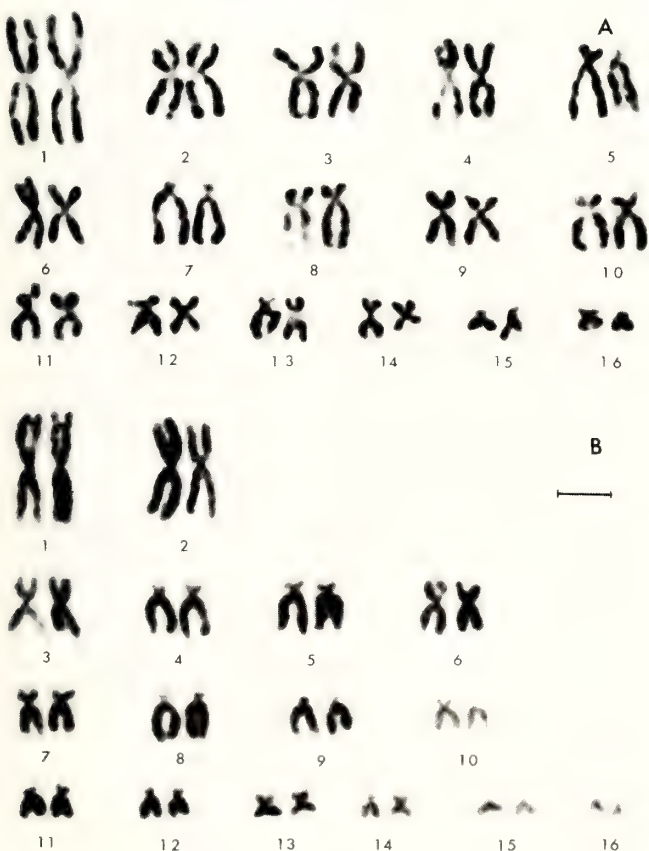


Fig. 3. Gymnosomata karyotypes (scale bar = 5 μ m). (A) *Pneumodermopsis paucidens*, (B) *Paraclione longicaudata*.

six selected spreads. The overall appearance of the karyotype (Fig. 3B), better visualized in the ideogram (Fig. 5), is very different from the previous species. Pair 1 represents the highest relative length of Gymnosomata species up to now investigated and is about nine times longer than the last pair. Pair 2 is also conspicuously larger than the remaining pairs whose size decrease progressively from pair to 14. Pairs 15 and 16 are very small (relative unit length 2.55 and 1.61, respectively). Morphology of the pairs is as follows: metacentric for pairs 1, 2, 3 and 6, submetacentric for pairs 7 and 13, subtelocentric-submetacentric for pair 14, subtelocentric for pairs 4, 5, 8, 9, 10, 11, 12, and telocentric for pairs 15 and 16. Metaphases were not successfully silver-stained in this species.

HETEROPODA

Due to the small number of individuals investigated in the following species, chromosome measurements were made only to determine chromosome morphology. Therefore, neither tables nor ideograms are given.

PTEROTRACHEA CORONATA

In this species, two females and one male were investigated. The female complement consists of $2n=32$. In nine cells studied, the female karyotype (Fig. 6A) shows two large metacentric, then five metacentric of decreasing size, then five submetacentric, one submetacentric-subtelocentric and one subtelocentric pairs of about the same size. For the male, the complement set consists of $2n=31$. In eight cells studied, the first large metacentric pair of the karyotype (Fig. 6B) is similar to that of the female. Next, heteromorphic chromosomes are present: a metacentric, a submetacentric-subtelocentric and a small telocentric suggesting the presence of sex chromosomes XY_1Y_2 . Thus, by comparison

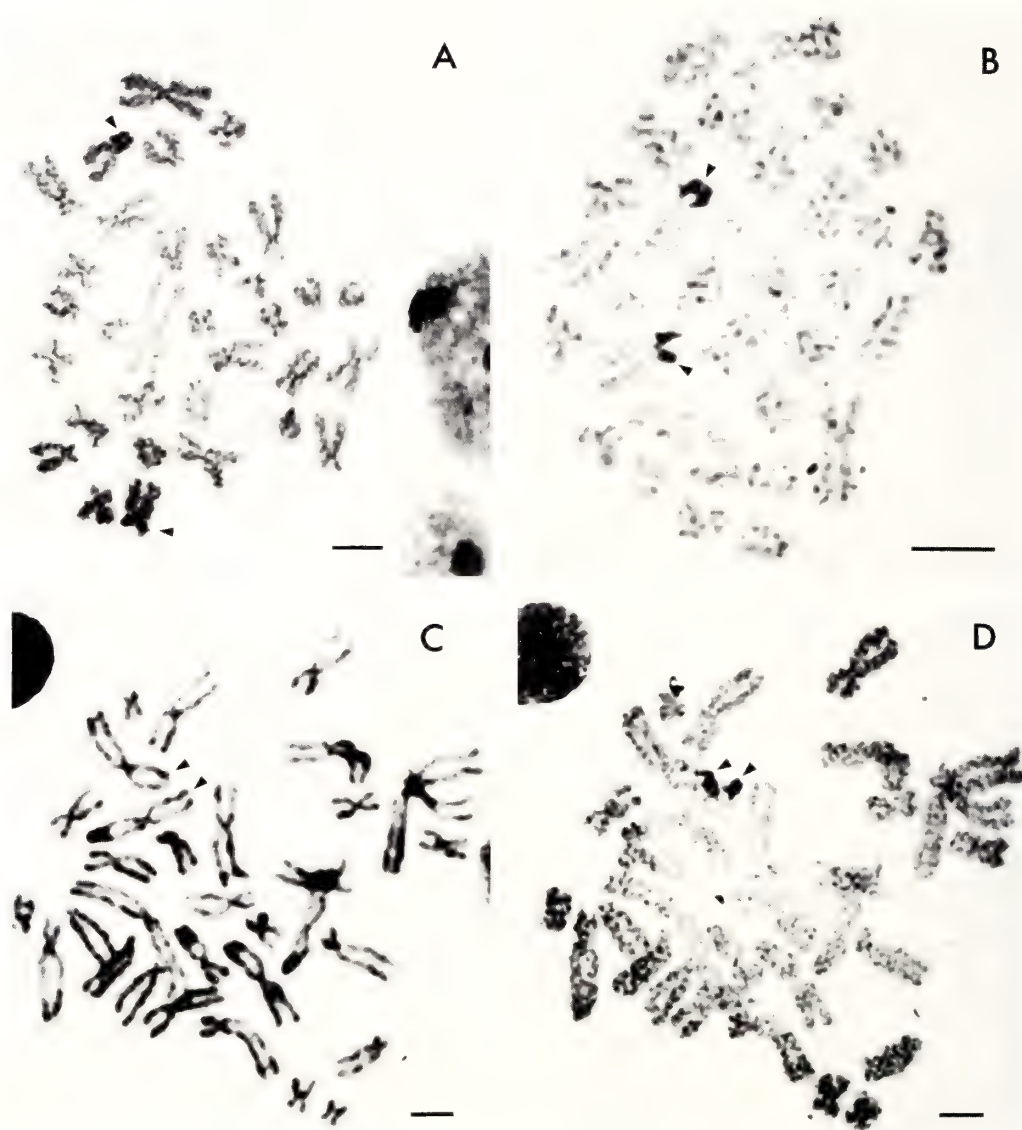


Fig. 4. Comparison of nucleolar organizer regions (NORs) in three Gymnosomata species (scale bar = 5 μ m). (A) silver-stained mitotic metaphase of *Pneumodermopsis canephora* (arrows point to NORs), (B) silver-stained mitotic metaphase of *Pneumoderma atlanticum* (arrows point to NORs), (C) Giemsa-stained mitotic metaphase of *Pneumodermopsis paucidens* (arrows on pair 2), (D) same metaphase, silver-stained (arrows on NORs).

the pair 2 of the female could be called XX. The remaining pairs in the male karyotype are similar to the female except the absence of the smallest autosome pair.

PTEROTRACHEA HIPPOCAMPUS

One male could only be investigated in this species. Sixteen well-spread metaphases were analysed with a complement set of $2n=31$. The karyotype (Fig. 7) shows heteromorphic chromosomes, comprising a large metacentric X, a telocentric Y_1 and a minute chromosome Y_2 , and 14 autosome pairs with two metacentric, two submetacentric, one subtelocentric-submetacentric, six subtelocentric and three telocentric.

FIROLOIDA DESMARESTI

In this species, several individuals of females and males were studied. But only two metaphasic cells in females and four in males could be karyotyped because of overlapping chromosomes. The overall large absolute chromosome size (Fig. 8A, B) of this species is probably the reason for this overlapping.

The female diploid set consists of $2n=32$ while the male shows $2n=31$. In both sexes, the first pair is a large metacentric. Next, the second metacentric pair in the female corresponds to XX chromosomes while in the male sex heteromorphic chromosomes are observed comprising a large metacentric X, a large submetacentric-subtelocentric Y_1 and a microchromosome Y_2 . The remaining autosome pairs in the

Table 3. Chromosome measurements and classification in six metaphasic cells of *Paraclione longicaudata*.

Chromosome pair no.	Relative length		Arm ratio		Centromeric index		Classification*
	Mean	SD	Mean	SD	Mean	SD	
1	15.15	0.725	0.877	0.055	46.65	1.554	m
2	11.78	0.864	0.751	0.119	42.07	3.642	m
3	8.17	0.497	0.733	0.121	41.47	3.315	m
4	7.99	0.346	0.227	0.025	18.44	1.645	st
5	7.45	0.352	0.265	0.112	20.32	6.303	st
6	7.04	0.593	0.768	0.115	42.26	3.444	m
7	6.59	0.439	0.366	0.070	26.45	3.667	sm
8	5.82	0.512	0.165	0.081	13.79	5.809	st
9	5.11	0.272	0.266	0.113	20.03	6.663	st
10	4.67	0.285	0.233	0.102	18.30	6.660	st
11	4.36	0.396	0.270	0.092	20.78	5.006	st
12	4.10	0.092	0.195	0.094	16.07	6.287	st
13	3.91	0.466	0.510	0.097	32.73	3.035	sm
14	3.85	0.542	0.363	0.202	24.84	10.174	st-sm
15	2.55	0.233	0.088	0.021	8.06	1.809	t
16	1.61	0.425	0.141	0.721	11.65	5.230	t

*m = metacentric; st = subtelocentric; sm = submetacentric; st-sm = subtelocentric-submetacentric; t = telocentric.

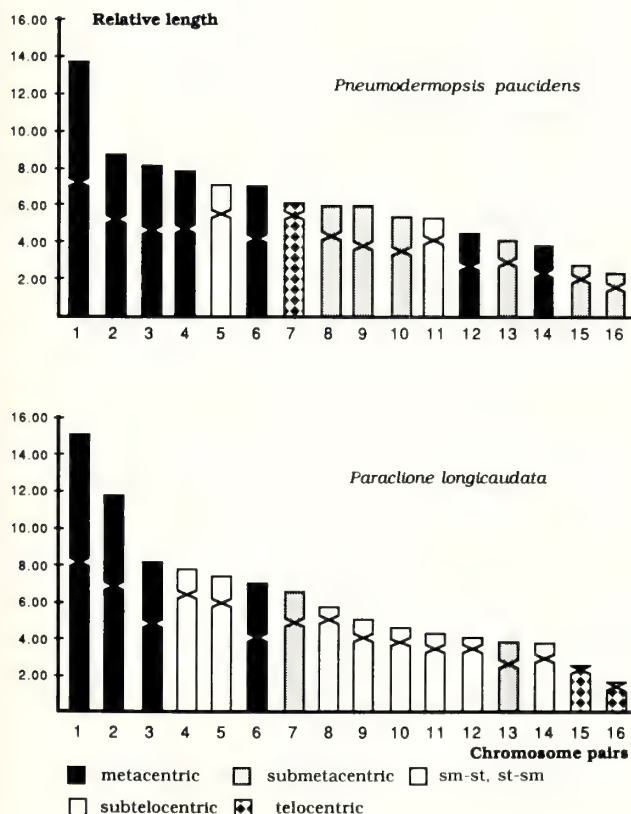


Fig. 5. Ideogram of *Pneumodermopsis paucidens* and *Paraclione longicaudata*, constructed from relative length and centromeric index values. Chromosome morphology is indicated: dark stippled for metacentric, medium stippled for submetacentric, light stippled for submetacentric-subtelocentric or subtelocentric-submetacentric, open for subtelocentric, lozenge stippled for telocentric.

female are four metacentric, four submetacentric, five subtelocentric and two telocentric. In the male, the smallest autosome pair is lacking.

DISCUSSION

THECOSOMATA

The diploid number of $2n=28$ observed in *Hyalocylis striata* is different from the number found by Zarnick (1911). This difference in counts for the same species may result from the difficulty in evaluating chromosome number using older cytological techniques. Comparing data obtained with the same techniques, however, points out that *H. striata* shows the highest diploid number among Euthecosomata.

Karyological data for all species studied in a previous paper (Thiriot-Quévèreux, 1988) and in the present work are recapitulated in table 4. The chromosome morphology of *Limacina inflata* (Orbigny), *Peraclis reticulata* (Orbigny) and *Cymbulia peroni* De Blainville is given following the nomenclature of Levan *et al.* (1964) after chromosome measurements.

The chromosomal features among Thecosomata could be assigned the following trends: (i) from low to high diploid number ($2n=20$ to $2n=28$ in Euthecosomata, and $2n=24$ to 34 in Pseudothecosomata); (ii) towards an increasing proportion of subtelocentric-telocentric chromosomes (zero to five pairs in Euthecosomata, and one to six Pseudothecosomata).

These features are unusual among Opisthobranchia. First, stable chromosome number within a family, or even an order, has been reported in many Opisthobranchia (Patterson, 1969). However, chromosome number variability of $2n=26$ to $2n=34$ have been observed within the Cephalaspidea order (Curini-Galletti, 1985, 1988), and of one bivalent within the Aplysiidae family (Vitturi *et al.*, 1982b) and

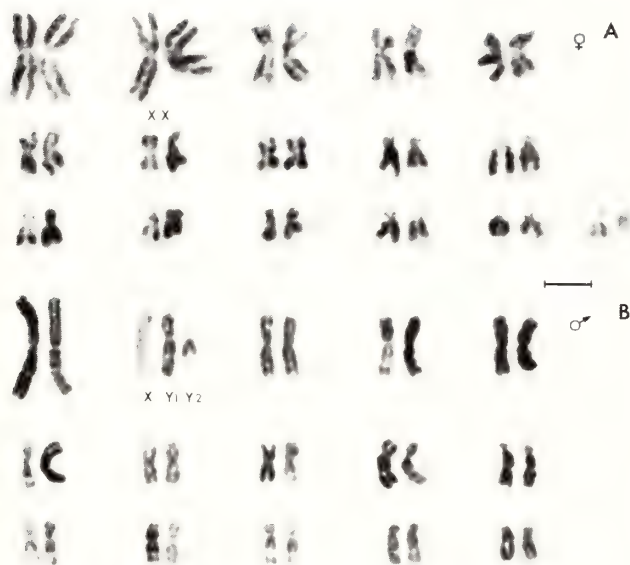


Fig. 6. Karyotypes of *Pterotrachea coronata* (scale bar = 5 μ m), (A), female, (B) male.

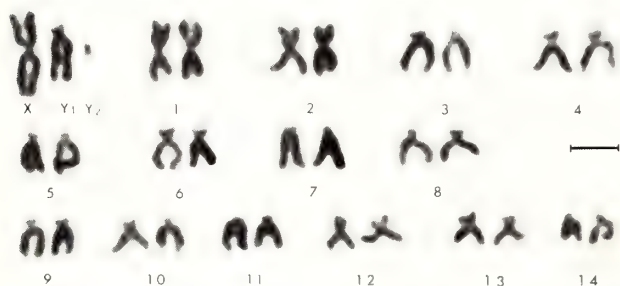


Fig. 7. Male karyotype of *Pterotrachea hippocampus* (scale bar = 5 μ m).

two Soleolifera families (see references in Patterson, 1969). Thus, the chromosome variability occurring among Thecosomata is especially large. Secondly, among the few karyological data recorded among Opisthobranchia, isobrachial chromosomes (i.e. metacentric or submetacentric) of a relatively homogeneous size are dominant in karyotypes (Curini-Galletti, 1988). Hence, Thecosomata differ greatly from the known Opisthobranchia. Moreover, if we assume that isobrachial chromosomes are plesiomorphic as suggested by Curini-Galletti (1988), the karyotypes of *Limacina inflata* showing only metacentric chromosomes should be considered as the most primitive. This point corresponds to the general agreement that the spirally coiled genus *Limacina* is the most primitive of the Euthecosomata (Lalli and Gilmer, 1989).

The evolutionary cytotaxonomy within Thecosomata could be from *Limacina inflata* with low diploid number and metacentric chromosomes to *Cymbulia peroni* with high diploid number and subtelocentric-telocentric chromosomes. The Thecosomata constitutes an isolated order with no karyological relationship with the other opisthobranch orders

and speculation about which ancestral molluscs might have given rise to the Thecosomata cannot be made.

GYMNOSOMATA

The two species studied in this paper share the same diploid number ($2n=32$) as the species previously investigated (Thiriott-Quévieux, 1988). This supports a chromosome number stability within Gymnosomata. This number is close to the opisthobranch ancestral number of $2n=34$ suggested by Schmeckel (1985). *Pneumodermopsis paucidens* reveals contradictory evolutionary features showing a plesiomorphic character (according to Curini-Galletti, 1988) with its highest proportion of isobrachial chromosomes, but also an apomorphic character (Thiriott-Quévieux, 1988 according to the hypothesis of Gold and Amemiya, 1986) with the presence of NORs on a metacentric chromosome. *Paraclione longicauda* differs from the other species by its striking variability in relative length of the different chromosomes and by its high number of subtelocentric chromosomes.

Therefore, karyological features among Gymnosomata are characterized by modal chromosome number of $2n=32$ and a variable proportion between metacentric-submetacentric and subtelocentric-telocentric chromosomes which, at present, preclude evolutionary implications.

In conclusion, karyological analyses of the two orders, Thecosomata and Gymnosomata, lend support for a biphyletical origin, i.e. different opisthobranch ancestors, as generally accepted (see Van der Spoel, 1967, and Lalli and Gilmer, 1989).

HETEROPODA

Since Boveri (1890) reported 32 chromosomes in the females of *Carinaria mediterranea* Blainville and *Pterotrachea mutica* Lesueur (= *P. hippocampus*), nothing has been published on the chromosomes of Heteropoda. The present data confirm a diploid complement of $2n=32$ in the females of *P. coronata* and *Firoloida desmaresti*. But the male diploid complement in the three species of Pterotracheidae here studied consists of $2n=31$ with heteromorphic chromosomes suggesting a XY_1Y_2 sex mechanism. The remaining autosome pairs of the Heteropoda karyotypes show a variable proportion of the different morphological types of chromosomes within the three species studied. The pelagic group of Heteropoda is considered as a superfamily (Boss, 1982), a suborder (Lalli and Gilmer, 1989) or an order (Van der Spoel, 1976) of the Prosobranchia Mesogastropoda. Chromosome number of $2n=14$ to $2n=34$ has been reported within Mesogastropoda, excluding polyploid species of Cerithiacea (see Patterson, 1969). In recent studies on Mesogastropoda, a chromosome number of $2n=32$ in Rissoidae (Thiriott-Quévieux and Ayraud, 1982), $2n=34$ in Littorinidae (Janson, 1983; Vitturi et al., 1986, 1988) and in Naticidae (Vitturi et al., 1982a; Komatsu, 1985) has been found. In the hydrobioid *Tricula aperta* (Temcharoen), diploid chromosome number showed variations from $2n=32$ to 34 in females and $2n=31$ to 33 in males (Kitikoon, 1982). Thus, the diploid complement observed in Heteropoda ranges close to these numbers.

Table 4. List of known karyological data for pelagic gastropods.

		Diploid no.	sex chromo.	Morphological types (no. of chromosome pairs of autosomes)					
				m	sm	sm-st st-sm	st	t	
Opisthobranchia									
Thecosomata, Euthecosomata									
	<i>Limacina inflata</i>	20		10					
	<i>Creseis acicula</i> (Rang)	20		5	5				
	<i>Creseis virgula</i> (Rang)	20		7	1	2			
	<i>Clio pyramidata</i> Linné	22		5	1	2	2	1	
	<i>Cavolinia inflexa</i> (Lesueur)	24		6	1	2	3		
	<i>Hyalocylis striata</i>	28		7	2	1	4		
Thecosomata, Pseudothecosomata									
	<i>Peraclis reticulata</i>	24		6	4	1	1		
	<i>Cymbulia peroni</i>	34		5	2	4	5	1	
Gymnosomata									
	<i>Pneumodermopsis canephora</i>	32		2	3	5	5	1	
	<i>Pneumodermopsis paucidens</i>	32		7	6	1	1	1	
	<i>Pneumoderma atlanticum</i>	32		3	5	4	4		
	<i>Paraclione longicaudata</i>	32		4	2	1	7	2	
Prosobranchia									
Heteropoda									
	<i>Pterotrachea coronata</i>	female	32	XX	7	5	1	2	
		male	31	XY ₁ Y ₂	7	4	1	2	
	<i>Pterotrachea hippocampus</i>	male	31	XY ₁ Y ₂	2	2	1	6	3
	<i>Firoloida desmaresti</i>	female	32	XX	4	4		5	2
		male	31	XY ₁ Y ₂	4	4		4	2

m : metacentric; sm : submetacentric; sm-st and st-sm : $23.06 < Ci > 26.74$; st : subtelocentric; t : telocentric.

**Fig. 8.** Karyotypes of *Firoloida desmaresti* (scale bar = 5 μ m), (A) female, (B) male.

Sex chromosomes have been observed in several species of Mesogastropoda with difference in male sex-determining mechanisms (YX vs. XO) (see references in Patterson, 1969; and Stern, 1975; Thiriou-Quievreux, 1982; Kitikoon, 1982; Vitturi *et al.*, 1988). Therefore, the XY₁Y₂ male sex mechanism here suggested in Pterotracheidae is very unusual among molluscs and probably involves translocations between sex chromosomes and autosomes (White, 1973). This sex mechanism, even if Y₂ is a microchromosome as in *Filoloida desmaresti* suggests a multiple sex-chromosome mechanism as reported by White (1973) in different taxons of the animal kingdom. However, further investigations on males and females of Heteropoda need to be carried out to confirm and elucidate this sex mechanism.

ACKNOWLEDGMENTS

Thanks are due to R. Voelksen for providing live animals from the plankton, to G. Quélart for her excellent technical assistance and to P. Chang for English corrections.

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Date of manuscript acceptance: 2 November 1989

SAMPLING REQUIREMENTS FOR EPIPELAGIC HETEROPOD MOLLUSCS

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ABSTRACT

Previous populational studies of epipelagic heteropods have demonstrated high between-replicate variability. Design of sampling programs whose objectives are the determination of temporal and/or spatial populational differences must address the consequences of such variability. Net size, the volume of water filtered by the net and the number of replicates necessary to sample the target populations are essential considerations. Because of the increase in sample volume, use of a large net would be expected to result in a decrease in between-replicate variability and an increase in sample precision. Large nets also reduce the likelihood of net avoidance and, therefore, the potential problem of underestimating species abundances.

The effect of sample volume on between-replicate variability in species densities was assessed by 30 min oblique tows to 50 m taken with 70 cm Bongo nets and a 226 cm ring net. The total volume of water filtered by the ring net averaged 3.6 times that filtered by the Bongo nets. Standard errors for the four most abundant species averaged 3.5 times greater for the Bongo nets than for the ring net. On average, the level of precision (ratio of standard error to mean) attained using the larger net (0.10) was almost twice that of the small net (0.18).

Estimates of the number of replicate tows needed to sample the abundant species of Hawaiian heteropods were determined from a series of 22 tows taken with the 226 cm ring net towed obliquely to a target depth of 300 m. Based on levels of precision (D) ranging from 0.10 to 0.20 and the mean (m) and variance (s^2) for each species from the 22 tows, the number of required replicates (n_r) was computed for each species using the equation: $n_r = s^2 m^{-2} D^{-2}$. Regression of the log variance and log mean for the eleven most abundant species had a high coefficient of determination (0.99). Based on this regression, variances can be predicted from sample means using Taylor's power law: $s^2 = 0.883m^{1.479}$.

For the seven most abundant species of heteropods estimates of the number of required replicates at five levels of precision were compared with plots of cumulative (or running) mean density and cumulative variance using the density data from the 22 oblique tows. The cumulative mean became stable at the number of replicate tows corresponding, on average, to a 0.15 level of precision. On the basis of these results ten replicate tows would be needed to sample the four most abundant species.

The heteropods are a holopelagic group of carnivorous gastropods that are highly mobile and locate their prey visually using well-developed eyes (reviewed by Lalli and Gilmer, 1989). Since the heteropods occupy a high trophic position as primary and secondary carnivores (Seapy, 1980; Richter, 1982), it is not surprising that they occur in low population densities. In the upper 100 m of the water column off southern California the carinarid heteropod *Carinaria japonica* Okutani, 1955, generally occurred at densities less than about 10 individuals per 1,000 m³, although the maximal density was 142 per 1,000 m³ (Seapy, 1974). Abundances of eleven species of heteropods from the equatorial eastern Pacific were reported by McGowan and Fraundorf (1966). The three most abundant species were atlantids that averaged between 124

and 225 animals per 1,000 m³ from eight oblique tows to 140 m taken with ring nets having mouth diameters of 1.0 and 1.4 m. The maximal density (598 individuals per 1,000 m³) was recorded for *Atlanta lesueuri* Souleyet, 1852. Species of epipelagic heteropods from Hawaiian waters (Seapy, 1990) generally averaged less than five individuals per 1,000 m³, although densities between 10 and 20 per 1,000 m³ were recorded for several species from shallower waters. In this study the maximal density was 58 *A. lesueuri* per 1,000 m³ between the surface and 50 m at night.

A feature common to the studies of both McGowan and Fraundorf (1966) and Seapy (1990) was high variability in species abundances between replicate tows. Since abundances of epipelagic heteropods are generally low and

between-replicate variability is high, an investigation of the sampling requirements for populations of these animals is needed and should be most beneficial to the planning of future sampling programs. The importance of such preliminary studies for the determination of optimal sample size and the number of required replicates has been widely acknowledged and was emphasized recently in a review of sampling by Andrew and Mapstone (1987).

METHODS

Epipelagic heteropods were collected from waters overlying the 2,000 m bottom depth contour at a distance of 11 to 20 km from the southwest coast of Oahu, Hawaii during two cruises of the R/V KILA, University of Hawaii in 1986. The effect of net size on between-replicate variability was investigated by comparing the densities of heteropod species from a series of six, shallow-water oblique tows taken with paired, 70 cm Bongo nets (0.8 m² combined mouth opening area) and a 226 cm ring net (4.0 m²). Three tows were taken with each type of net to a target depth of 50 m between 1031 and 1622 hrs on 23 November 1986. Tow duration averaged 30 min, and the towing speed was 2-3 knots. The ring net attained a maximal depth of 50 m during each tow and filtered an average of 5,112 m³ (range = 4,508-5,841 m³), while the Bongo nets achieved an average maximal depth of 40 m (range = 38-42 m) and filtered an average of 1,417 m³ (range = 1,086-1,700 m³). Thus, net size differed while tow duration was the same, resulting in a sample volume that was, on the average, 3.6 times greater for the ring net than for the combined Bongo nets. For each net type replicate variability was expressed in terms of the standard error (standard deviation divided by the square root of the number of samples). Sampling precision (the ratio of the standard error to the mean) was determined for the four most abundant species (mean densities greater than 4.0 individuals per 1,000 m³) for the two types of nets.

The numbers of replicate tows required to sample heteropod populations were investigated using a series of 22 oblique tows to a target depth of 300 m taken with a 226 cm ring net (mouth opening = 4.0 m², mesh width = 0.5 mm). Tows were taken consecutively over a 24 hr period between 1524 hrs on 24 March and 1539 hrs on 25 March 1986, resulting in a total of 11 day and 11 night tows. The duration of the tows ranged from 32 to 43 min and averaged 38 min. The volume of water filtered by the net during each tow was determined using a T.S.K. Model OI-210 Flow Meter, and averaged 6,973 m³ (standard deviation = 501 m³, range = 5,820 to 7,963 m³). The maximal depth achieved during each tow was obtained from a Benthos Time-Depth Recorder. Although the maximal target depth was 300 m, actual depths ranged from 240 to 400 m (average depth = 299 m; standard deviation = 37 m).

Based on vertical distribution and abundance data from an earlier study (Seapy, 1990), greater than 99% of the epipelagic heteropods off Hawaii occur in waters shallower than 200 m (which represents the upper one-half of the epipelagic zone off Hawaii; Young, *et al.*, 1980). Thus, oblique

tows that extend deeper than 200 m can be considered to have adequately sampled the vertical range of the Hawaiian epipelagic heteropods. Since actual species abundances (expressed as numbers per unit volume) differ both vertically and, for deeper-dwelling species, between day and night periods (Seapy, 1990), densities are most appropriately expressed in terms of numbers beneath a unit area of sea surface (area sampled = volume filtered/maximal depth of tow). In the present study the area sampled ranged from 15.4 to 31.9 m² per tow, and the mean area was 23.7 m². To standardize these differences in areas sampled, all density data were converted to numbers beneath 25.0 m² of sea surface.

The numbers of replicates needed to sample the seven most abundant species of heteropods collected by the 22 oblique tows taken in March were determined using a method described by Elliott (1971) for application with the freshwater benthos. This method has been used subsequently in several studies, e.g. McIntyre *et al.* (1984) and Vezina (1988) for the marine benthos and by Downing *et al.* (1987) for marine and freshwater zooplankton. The general formula is:

$$n_r = s^2 m^{-2} D^{-2} \quad (1)$$

where: n_r = number of required replicates; s^2 = sample variance; m = sample mean; D = precision (ratio of the standard error to the mean). To apply this equation the investigator must select a required level of precision (D). This decision is of practical importance because the number of required replicates increases sharply as the level of precision increases. Elliott indicated that one should be able to tolerate a standard error equal to 20% of the mean (i.e. $D = 0.20$) for freshwater benthic populations. However, if one required a standard error equal to 10% of the mean (i.e. $D = 0.10$), the number of replicates would have to be four times as great.

In addition to selecting the level of precision, equation (1) requires estimates of sample means and variances. A limited number of preliminary tows can give an approximate range of mean densities for the species under consideration. However, since high between-replicate variability has been reported (McGowan and Fraundorf, 1966; Seapy, 1990) for the epipelagic heteropods, high variances would be expected. Since a greater number of replicate samples appears to be necessary to make more reliable estimates of the variance (see Results), it would be beneficial if one could predict variance values based on means that had been obtained from a relatively small number of preliminary samples. For a variety of natural populations the mean has been shown (Downing, 1979; Downing and Cyr, 1985; Morin, 1985; Downing *et al.*, 1987; Vezina, 1988) to be related to the variance by the power law of Taylor (1961):

$$s^2 = am^b \quad (2)$$

where: s^2 = sample variance; m = sample mean; a = a constant (the value of s^2 corresponding to a mean of 1.0, determined from the regression equation); b = the regression coefficient (i.e. the slope of the regression line). In the present study log mean was plotted against log variance for the eleven most abundant species sampled by the 22 oblique tows in March 1986. Values for the constants a and b in

equation (2) were obtained from a least squares regression of log mean on log variance.

The density data from the 22 oblique tows were also used to calculate and plot cumulative (or running) means and cumulative (or running) variances for the seven most abundant species. The number of required replicates corresponding to five levels of precision (0.10, 0.125, 0.15, 0.175 and 0.20) were indicated on each cumulative mean plot for visual comparison with the cumulative mean and cumulative variance data.

RESULTS

Densities of the four most abundant species of heteropods from samples collected with 70 cm Bongo nets and a 226 cm ring net to a target depth of 50 m (Table 1) were not significantly different (Mann Whitney U test). Replicate variability resulting from the use of the two types of nets was assessed by computing standard errors (Fig. 1), which averaged 3.5 times greater for the Bongo nets than for the ring net and ranged from 1.8 (*Firoloida desmaresti* Lesueur, 1817) to 5.0 (*Atlanta oligogyra* Tesch, 1906) times greater for the Bongo nets. The level of sampling precision (Table 1) was, on average, almost twice as great for the ring net (mean D = 0.10) as for the Bongo nets (mean D = 0.18), which corresponds to a nearly four-fold increase in replication requirements.

A total of thirteen species of epipelagic heteropods were represented among the 22 oblique tows taken in March 1986 (Table 2). For the seven most abundant species (those with mean densities greater than four per 25 m²), no significant density differences (Mann Whitney U test) were obtained between the 11 day and 11 night tows (Table 3). Thus, the day and night data were pooled in the subsequent analyses.

A log-log plot was constructed (Fig. 2) from the mean and variance values computed for the eleven most abundant species collected by the 22 replicate tows (Table 2). The resultant regression line was described by the equation:

$$\log s^2 = -0.125 + (1.479) (\log m) \quad (3)$$

and had an extremely high coefficient of determination ($r^2 = 0.99$), which was highly significant ($t = 31.9$; $p < .01$). Based on this regression analysis the relationship between the heteropod means and variances can be expressed by Taylor's (1961) power law:

$$s^2 = 0.883m^{1.479} \quad (4)$$

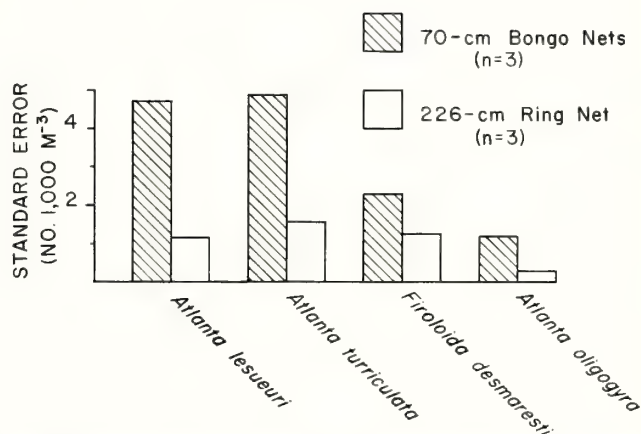


Fig. 1. Standard errors (numbers per 1,000 m³) for the four most abundant species of heteropods collected by oblique tows to a target depth of 50 m using 70 cm Bongo nets and a 226 cm ring net.

The numbers of replicates required to sample the seven most abundant species were estimated (Table 4) at five different levels of precision, ranging from 10% to 20%, using the mean and variance values for the 22 replicate tows in Table 2. For comparison, the numbers of required tows were also computed for each species using variances determined from equation (4). Numbers of required replicates ranged from four to ten at the 20% level of precision, and from 14 to 40 at the 10% level (Table 4). These data illustrate the principle that an increase in the level of precision from 20% to 10% results in a four-fold increase in the number of required replicates. For this reason selection of an appropriate level of precision is of great importance in the design of a sampling program.

The numbers of required replicate tows corresponding to each of the five levels of precision in Table 4 were included on cumulative mean plots for the five most abundant species (Figs. 3-7). The level of precision which appeared to be most appropriate for each species was then assessed visually from the plots. For *Atlanta peroni* (Fig. 3) the cumulative mean became relatively stable and approximated the final mean value of 32.8 per 25 m² (indicated by the horizontal line in the plot) after the seventh tow. On the cumulative mean plot the eighth tow falls between the number of required tows at the 0.15 and 0.125 levels of precision. If one had only taken eight tows, however, the variance would have been overestimated by nearly 25% (the final variance

Table 1. Mean densities (numbers 1,000 m⁻³), standard errors (SE) and precisions (D) for the four most abundant species captured by replicated oblique tows to a target depth of 50 m on 23 November 1986 using paired 70 cm Bongo nets and a 226 cm ring net.

Species	Bongo Net tows (n = 3)			Ring Net tows (n = 3)		
	Mean	SE	D	Mean	SE	D
<i>Atlanta lesueuri</i>	39.0	4.7	0.12	24.7	1.1	0.04
<i>Firoloida desmaresti</i>	11.5	2.3	0.20	10.3	1.3	0.13
<i>A. turriculata</i> d'Orbigny, 1836	16.5	4.8	0.29	8.2	1.5	0.18
<i>A. oligogyra</i>	9.6	1.2	0.13	4.1	0.2	0.05

Table 2. Mean densities, ranges and variances (numbers 25 m⁻²) of epipelagic heteropods captured by 22 replicate oblique tows to a target depth of 300 m on 24-25 March 1986.

Species	Mean	Range	Variance
<i>Atlanta peroni</i> Lesueur, 1817	32.8	10.6 - 52.7	158.0
<i>A. plana</i> Richter, 1972	25.5	11.4 - 43.5	95.1
<i>A. inflata</i> Souleyet, 1852	18.4	8.9 - 37.3	54.4
<i>Protatlanta souleyeti</i> (Smith, 1868)	13.5	1.3 - 25.8	41.1
<i>A. turriculata</i>	8.7	2.7 - 21.3	18.8
<i>A. lesueuri</i>	5.1	0.8 - 11.4	10.3
<i>A. meteori</i> Richter, 1972	4.6	0 - 9.5	6.4
<i>A. helicinoides</i> Souleyet, 1852	2.3	0 - 6.1	3.0
<i>A. oligogyra</i>	1.3	0 - 6.5	2.3
<i>A. fusca</i> Souleyet, 1852	1.1	0 - 4.2	1.0
<i>A. tokiokai</i> van der Spoel and Troost, 1972 (= <i>A. inclinata</i> Souleyet, 1852)	0.9	0 - 3.7	1.2
<i>A. echinogyra</i> Richter, 1972	0.1	0 - 1.4	0.1
<i>Oxygyrus keraudreni</i> (Lesueur, 1817)	0.1	0 - 1.1	0.1

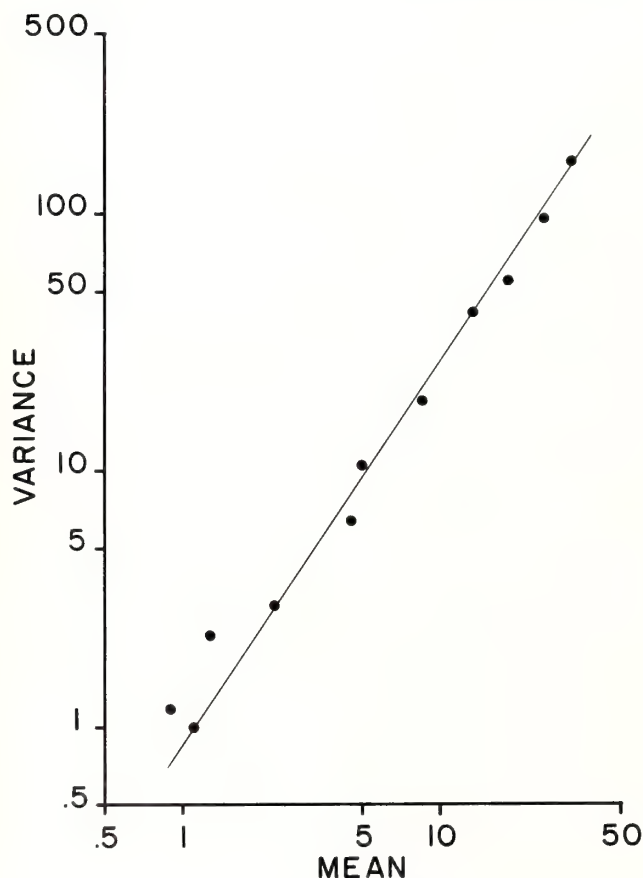


Fig. 2. Log-log plot of sample mean and variance for eleven species of heteropods obtained from 22 oblique tows to a target depth of 300 m. The regression equation for the plotted line is: $\log_{10} \text{variance} = -0.125 + (1.479) (\log_{10} \text{mean})$.

value is indicated by the horizontal line in the plot). The cumulative variance declined to about the level of the final variance value at the eleventh tow, decreased and then increased gradually after that point. The plot of the cumulative mean for *A. plana* (Fig. 4) was similar to that for the preceding species. The cumulative mean approached the final mean value at the eighth tow and was very stable beyond that point. The eighth tow corresponded to the number of required replicates between the 0.15 and 0.125 levels of precision. The cumulative variance "overshot" the final variance by 33% at the eighth tow and did not decrease to the level of the final variance until the eighteenth tow.

The cumulative mean for *Atlanta inflata* (Fig. 5) approximated the final mean value and remained nearly constant after the thirteenth tow. However, beginning with the seventh tow (corresponding to the 0.15 level of precision), the cumulative mean changed only gradually. Like *A. plana*, the cumulative variance "overshot" the final variance at the seventh tow, and then remained above the level of the final variance until the eighteenth tow. The cumulative mean for *Protatlanta souleyeti* (Fig. 6) decreased slowly between the fifth and twelfth tows, after which it gradually rose to nearly the level of the final mean value by the eighteenth tow. However, the cumulative mean was close initially to the final mean value between the seventh and tenth tows (corresponding to the 0.175 and 0.15 levels of precision), and changed only gradually after the seventh tow. The cumulative variance "overshot" (at four tows) and then "undershot" (at ten tows) the final variance, but became relatively stable at levels near the final variance after the eleventh tow.

The plots of cumulative mean and variance for *Atlanta turriculata* (Fig. 7) were quite different from those of the preceding species. The cumulative mean remained below the

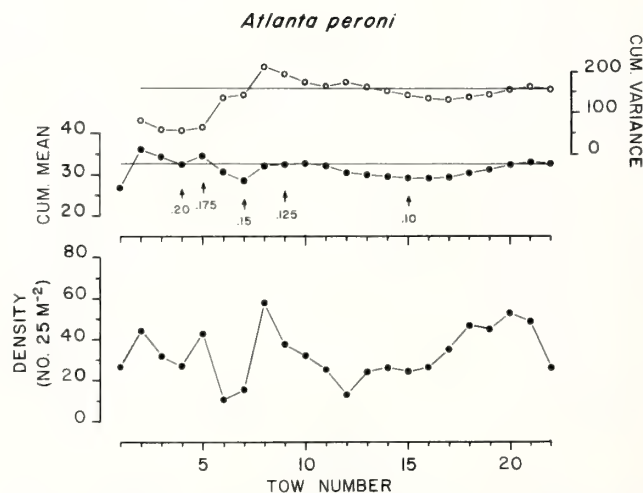


Fig. 3. Densities (numbers per 25 m²) of *Atlanta peroni* from each of the 22 oblique tows taken to a target depth of 300 m (lower illustration). Plots of the cumulative sample mean and cumulative sample variance in numbers per 25 m² (upper illustration). The number of required replicates corresponding to five levels of precision (0.20, 0.175, 0.15, 0.125, and 0.10) are included on the plot of the cumulative mean.

Table 3. Comparison of species densities (numbers 25 m⁻²) between day and night periods for the seven most abundant epipelagic heteropods captured 24-25 March 1986 off Oahu. Day-night differences in densities were tested by the Mann Whitney U statistic (s = significant at p < .05; ns = not significant).

Species	Day (n = 11)		Night (n = 11)		Signif.
	Mean	Variance	Mean	Variance	
<i>Atlanta peroni</i>	37.3	104.0	28.4	172.4	ns
<i>A. plana</i>	24.5	75.0	26.5	113.3	ns
<i>A. inflata</i>	18.4	26.9	18.5	81.8	ns
<i>Protatlanta souleyeti</i>	16.5	36.3	10.4	27.6	ns
<i>A. turriculata</i>	9.6	22.9	7.8	13.0	ns
<i>A. lesueuri</i>	5.0	8.4	5.3	12.2	ns
<i>A. meteori</i>	4.6	4.6	4.7	8.3	ns

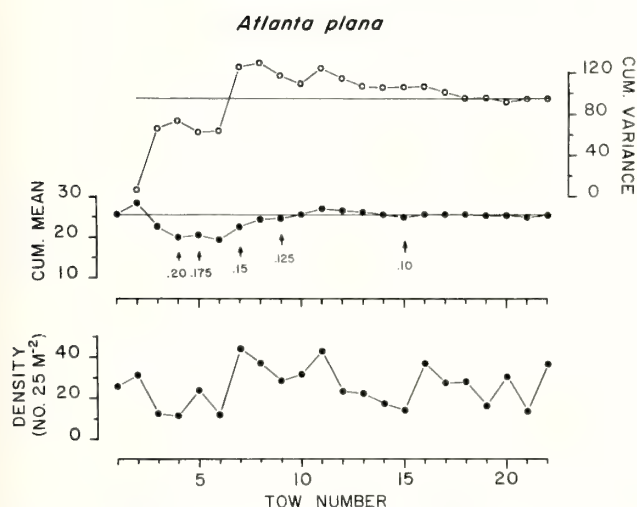


Fig. 4. Densities (numbers per 25 m²) of *Atlanta plana* from each of 22 oblique tows to a target depth of 300 m (lower illustration). Plots of the cumulative sample mean and cumulative sample variance in numbers per 25 m² (upper illustration). The number of required replicates corresponding to five levels of precision (0.20, 0.175, 0.15, 0.125 and 0.10) are included on the plot of cumulative mean.

final mean value between the second and ninth tows. At the tenth tow (corresponding to the number of required replicates between the 0.175 and 0.15 levels of precision), the cumulative mean approximated the final mean value and changed only gradually thereafter. The cumulative variance was far below the final variance (by a maximum of 94% at the sixth tow) until the twentieth tow.

Cumulative mean and variance plots were also constructed for *Atlanta lesueuri* and *A. meteori*, but are not included here. The cumulative mean plot for *A. lesueuri* oscillated between 6.0 (at the second tow), 3.8 (at the fifth tow) and 6.0 individuals per 25 m² (at the tenth tow). After the eighth tow, however, the cumulative mean decreased gradually to a value close to the final mean at the fourteenth tow (corresponding to the number of required replicates between the 0.15 and 0.175 levels of precision). The cumulative variance followed a similar pattern to the cumulative mean, but was extremely stable and approximated the final variance after the tenth tow. For *A. meteori* the cumulative mean decreased from

Table 4. The numbers of replicates required to sample the seven most abundant species of epipelagic heteropods at five levels of precision, using equation (1) from the text and the species means and variances (n = 22 tows) from Table 1. The values in parentheses are the numbers of required replicates based on means from Table 1 and variances computed from equation (4).

Species	Level of Precision				
	0.10	0.125	0.15	0.175	0.20
<i>Atlanta peroni</i>	15 (14)	9 (9)	7 (6)	5 (5)	4 (4)
<i>A. plana</i>	15 (16)	9 (10)	7 (7)	5 (5)	4 (4)
<i>A. inflata</i>	16 (19)	10 (12)	7 (9)	5 (6)	4 (5)
<i>Protatlanta souleyeti</i>	23 (23)	14 (15)	10 (10)	7 (7)	6 (6)
<i>A. turriculata</i>	25 (29)	16 (18)	11 (13)	8 (9)	6 (7)
<i>A. lesueuri</i>	40 (38)	25 (24)	18 (17)	13 (12)	10 (9)
<i>A. meteori</i>	30 (40)	19 (26)	13 (18)	10 (13)	8 (10)

initial high values of 6.5 per 25 m² (the second through fourth tows) to a value close to the final mean at the eleventh tow (corresponding to the number of required replicates between the 0.15 and 0.175 levels of precision), and remained stable thereafter. Cumulative variance values were above (by a maximum of 72%) the level of the final variance between the fifth and twenty-second tows.

The above analyses of the cumulative mean plots suggest that the number of required replicate tows for the included heteropod species correspond, on average, to about the 0.15 level of precision. Inspection of the required number of replicates at a precision of 0.15 in Table 4 indicates that one would have to take ten replicate tows in order to sample the four most abundant species, while about eighteen tows would be required if one were to include all seven of the most abundant species.

The above comparisons of the cumulative mean and cumulative variance plots (Figs. 3-7) indicated that for most species the cumulative mean stabilized at a much lower

number of tows than did the cumulative variance. Thus, although a preliminary sampling program for heteropod species populations would provide adequate estimates of species mean densities, the sample variances are likely to be inaccurate. Instead, the variances corresponding to the sample means should be determined from equation (4).

DISCUSSION

A preliminary sampling program is required when the research objectives include spatial and/or temporal comparisons of population densities. The present study suggests that the characterization of zooplankton populations at a particular place and time can be accomplished by taking replicated oblique tows that extend through the vertical ranges of the species under investigation. Preliminary samples are necessary for the determination of the appropriate sample size (in this case, the size of the net and the volume of water to be filtered by the net) and the number of required replicates. These considerations are extremely important in oceanographic studies because of the difficulty in obtaining working time aboard research vessels and the high cost of ship time. Even if sampling time is not limiting, one must also take into account the costs and time required to process the collected samples. The relationship between sampling efficiency and costs have been discussed for marine populations in general by Andrew and Mapstone (1987) and for the zooplankton in particular by Cassie (1968), Wiebe (1971) and Downing *et al.* (1987).

Because heteropods occur in relatively low abundance, adequate numbers of specimens and representation by the species present in an area can best be achieved by filtering a relatively large volume of water during each tow. Large plankton nets, such as the 226 cm ring net used here, are preferred over smaller nets since they can filter a large volume of water in a relatively short time period. Large nets also

reduce the sampling bias resulting from possible net avoidance and improve the chances that the less abundant species will be adequately represented (McGowan and Fraundorf, 1966; Cassie, 1968). Between-replicate variability is reduced and sampling precision is increased by using large nets (Wiebe, 1971), and the increased sample volume can result in a substantial decrease in sample variance and, thus, in the number of required replicates (e.g. Andrew and Mapstone, 1987; Downing *et al.*, 1987). These relationships were supported by the present study, in which a reduction of between-replicate variability by two to five-fold and an in-

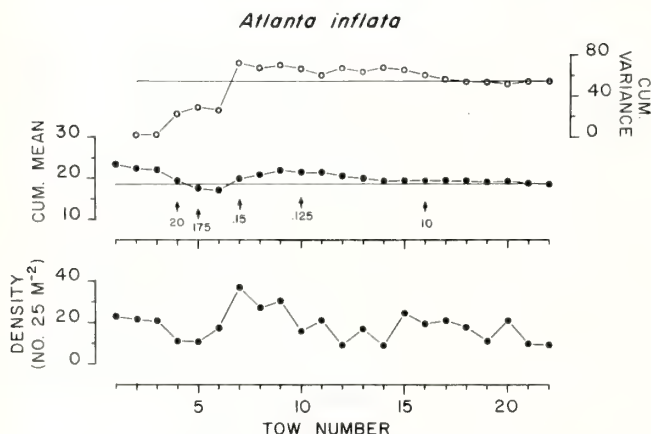


Fig. 5. Densities (numbers per 25 m²) of *Atlanta inflata* from each of 22 oblique tows to a target depth of 300 m (lower illustration). Plots of the cumulative sample mean and cumulative sample variance in numbers per 25 m² (upper illustration). The number of required replicates corresponding to five levels of precision (0.20, 0.175, 0.15, 0.125 and 0.10) are included on the plot of cumulative mean.

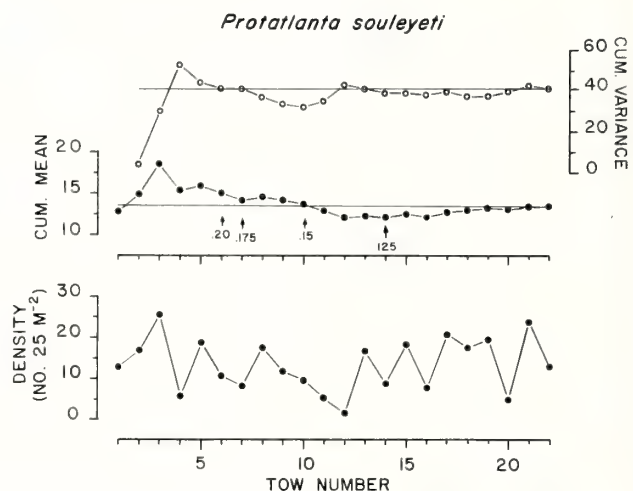


Fig. 6. Densities (numbers per 25 m²) of *Protatlanta souleyeti* from each of 22 oblique tows to a target depth of 300 m (lower illustration). Plots of the cumulative sample mean and cumulative sample variance in numbers per 25 m² (upper illustration). The number of required replicates corresponding to four levels of precision (0.20, 0.175, 0.15 and 0.125) are included on the plot of cumulative mean.

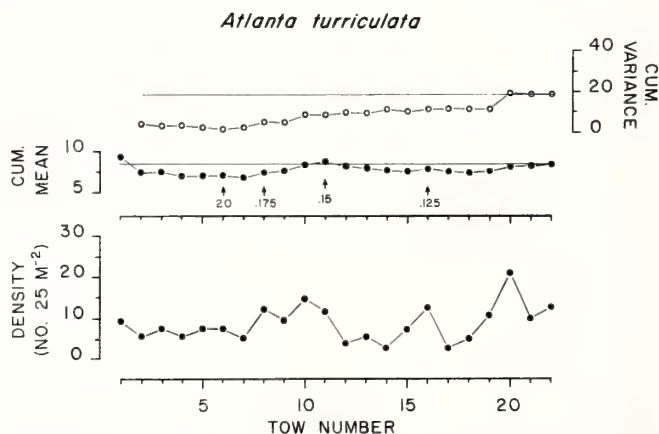


Fig. 7. Densities (numbers per 25 m²) of *Atlanta turriculata* from each of 22 oblique tows to a target depth of 300 m (lower illustration). Plots of the cumulative sample mean and cumulative sample variance in numbers per 25 m² (upper illustration). The number of required replicates corresponding to four levels of precision (0.20, 0.175, 0.15 and 0.125) are included on the plot of cumulative mean.

crease in sampling precision that averaged nearly two-fold resulted from sampling with a 226 cm ring net compared with 70 cm Bongo nets. Reduction in the numbers of required replicate tows provides an added benefit of reducing the time period necessary for the collection of the samples. Even if one makes a conscious effort to "track" the body of water being sampled (such as McGowan and Fraundorf, 1966, did by using a reference drogue), the chances of sampling the same parcel of water decrease as the total sampling time increases.

Analyses of the cumulative plots of species means and variances (Figs. 3-7) indicated that the numbers of replicate tows necessary to estimate the final variance values (after 22 tows) were higher (by several to many samples) than were needed to estimate the final means. Thus, the direct computation of variances from preliminary sample means using Taylor's power law is recommended. Taylor's power law has been applied to mean and variance data for populations from a variety of different habitats. For marine benthic populations Vezina (1988) tabulated over 3,000 estimates of mean and variance and showed that although the data were obtained from populations in varied types of habitats, the least squares regression of log mean on log variance resulted in a high coefficient of determination (0.86). The variance could then be predicted from the mean using Taylor's power law ($s^2 = am^b = 1.641 m^{1.219}$). The value of b (1.219) was lower than the average of about 1.5 found for freshwater benthic populations (Downing, 1979; Downing and Anderson, 1985; Downing and Cyr, 1985). Cassie (1963) reported a value for b of 1.57 for zooplankton populations. The b value from the present study (1.479) is somewhat lower than these last two values, and is considerably lower than that (1.849) obtained by Downing *et al.* (1987) from an analysis of 1,189 sets of replicate samples of freshwater and marine zooplankton. The value for b obtained by Downing *et al.* (1987) was based on a variety of sources whose density data ranged from 5×10^{-7} to 1.6×10^3 individuals per liter and sample values that ranged from 0.8 to 10^6 liters. To standardize these varied data, species densities were transformed to numbers of individuals per liter. By applying a term for sample volume (V_c) in the equation for Taylor's power law ($s^2 = am^bV_c$), Downing *et al.* reduced the value of b to 1.622, which is much closer to that obtained in the present study.

The results of Vezina's (1988) analyses of the mean to variance relationship argue that abundance data should be given in units appropriate to the sample size used in the study. Expression of the abundance data from the present study in terms of numbers per 25 m² was done in order to approximate the average area sampled by the 22 replicate tows (i.e., 23.7 m²). In turn, this resulted in density values that were close to the raw sample counts, e.g., the mean density of *Atlanta peroni* was 32.8 individuals per 25 m² and the average number of *A. peroni* collected per tow was 30.8. Density data (in numbers per 1,000 m³) were tabulated for eight species of atlantids from the eastern Pacific Ocean (southeast tip of Baja California) by McGowan and Fraundorf (1966). Four series of oblique tows to 140 m were taken with ring nets of different sizes. However, possible avoidance of all nets except for the

largest one (140 cm diameter) was suggested for several species. The average volume of water filtered by the four tows taken with the 140 cm net was 510 m³, and I converted their data from numbers per 1,000 m³ to numbers per 500 m³ for analysis here. Regression of log mean and log variance for the six most abundant species (range in mean density = 1.5 for *A. fusca* to 111.1 animals per 500 m³ for *A. gaudichaudi* Souleyet, 1852) resulted in the regression equation, $\log s^2 = -0.666 + (1.567)(\log m)$, which had a very high coefficient of determination ($r^2 = 0.94$). Expressed as Taylor's power law, $s^2 = 0.515m^{1.567}$. Superimposing this regression line on figure 2 results in remarkably close agreement (in terms of both the slope and elevation) with the regression for the eleven species of Hawaiian heteropods. The value of b (1.567) is only slightly greater than that found in this study (1.479) and matches the value of 1.57 reported by Cassie (1963).

In conclusion, the present results suggest that the four most abundant species of epipelagic heteropods off Hawaii (i.e. those species whose densities exceeded 13 individuals per 25 m²) can be sampled by ten replicate oblique tows to a target depth of 300 m using a continuously-open, 226 cm ring net. To sample the three most abundant species (i.e. those whose mean densities exceed 18 per 25 m²) fewer tows (eight) would be needed, while considerably more tows (about 18) would be required to sample the sixth and seventh most abundant species (mean densities of about five per 25 m²). Application of Taylor's power law to the mean and variance data for the eleven heteropod species sampled by the ring net indicated that variances can be predicted with a high degree of confidence from sample means (equation 4). Thus, the number of required replicates for a sampling program whose goal is the spatial and/or temporal comparison of heteropod species populations can be determined using equation (1) from estimates of population means, their corresponding variances (from equation 4) and the assumption of a 15% level of precision.

ACKNOWLEDGMENTS

The samples used in this study were collected during two cruises in 1986 of the R/V KILA, University of Hawaii. I am most grateful for the support of the officers, crew and members of the scientific party during these cruises, and to Richard Young for his collaboration in the sampling program. For the loan of the Bongo nets, I thank Jed Hirota. My appreciation is extended to Richard Young and Jed Hirota for their critical reviews of the manuscript. This study was supported by National Science Foundation Grant OCE-8500593.

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Date of manuscript acceptance: 25 November 1989.

IN SITU OBSERVATIONS OF FEEDING BEHAVIOR OF THECOSOME PTEROPOD MOLLUSCS

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ABSTRACT

Recent *in situ* observations of thecosome pteropods were made during five cruises in tropical, temperate, and arctic waters of the North Atlantic Ocean, and during one austral summer season in Antarctic waters. The long quiescent periods employed by thecosomes to fish their mucous feeding webs and the apparent lack of a pumping mechanism to move water through the web suggests they rely on contact trapping of large, motile prey. Species of *Cavolinia* and *Diacria* were estimated to remain in one location for at least 35 min to fish sequential webs. The external spherical webs used by euthecosomes and their ability to rapidly ingest them appear to be a unique feeding method among plankton that utilize mucous feeding structures. All euthecosomes observed during night dives entrapped numerous small crustaceans as the webs were withdrawn, and all specimens diver-collected at night contained small copepods in their guts. Crustaceans also accounted for up to 25% by volume of the gut contents of *Limacina helicina* (Phipps) preserved *in situ* in arctic waters during July and August, 1988. Individuals of a given species use webs of comparable dimensions in different water masses. Based on the observed feeding strategies and on the common ingestion of copepods by arctic *Limacina* and by temperate and tropical cavoliniid species, carnivory can not be precluded as a primary feeding habit for the Thecosomata.

The Thecosomata comprise an order of common opisthobranch gastropods which exist in the holoplankton by means of parapodia for rapid swimming and by the use of large, external mucous webs to collect food (Lalli and Gilmer, 1989). Their feeding habits depend on buoyancy control and passive drifting. Consequently, they have greatly reduced the wall thickness of their external shell or replaced it with internal gelatinous conchae. They have no gills which can function for food gathering and resemble vermetid prosobranchs (Hughes and Lewis, 1974; Hughes, 1978) by using cilia on the surfaces of the mantle and footlobes to manipulate their feeding webs. Much of their behavior remains obscure due to their remote habitat, and the difficulty of observing them in an undisturbed state. In addition, their fragility makes collection of undamaged specimens difficult. Even carefully collected individuals display abnormal behavior in the laboratory where they only survive for brief periods.

Using blue water scuba techniques (Hamner, 1975) to observe undisturbed thecosomes has been the most useful means to study their feeding habits. The feeding webs are usually so fragile and transparent that they are only visible in daylight with bright strobe lighting or by the delicate

application of carmine particles to the web surfaces (Gilmer and Harbison, 1986). The euthecosomes (*Limacina*, *Creseis*, *Styliola*, *Hyalocylis*, *Clio*, *Cavolinia*, *Diacria*) use spherical webs attached directly to the ciliated footlobes on the wings. The pseudothecosome genera (*Peraclis*, *Cymbulia*, *Corolla*, *Gleba*) use large flat or funnel shaped webs that float above the wingplate and are attached to the animal by a proboscis composed of the footlobes. Thecosomes are extremely sensitive to turbulence and, at the slightest provocation, will abandon their feeding activity with rapid escape swimming. Although the abandoned webs are left floating intact, their transparent and fragile nature have thus far made it impossible to sample them quantitatively.

Information on thecosome diet is limited to a few qualitative descriptions of gut contents, fecal pellets, and web fragments (see review in Lalli and Gilmer, 1989). Based on these studies, thecosomes appear to be indiscriminate omnivores ingesting all size categories of prey from 1 μ m bacteria to copepods as large as 3 mm in length [seen in *Limacina heliocoides* Jeffreys, Gilmer (pers. obs.)]. Thecosomes, however, are often categorized solely as herbivores (e.g. Morton, 1954; Silver and Bruland, 1981; Foster,

1987; Boysen-Ennen and Piatkowski, 1988) since many phytoplankton cells are captured and ingested with the web, and are prevalent in the fecal pellets. Herbivory is also considered synonymous with mucous suspension feeding (Jorgensen, 1966). This label, however, ignores a much broader diet that often includes many protozoan and zooplankton prey items (e.g. Richter, 1977, 1983; Ishimaru *et al.*, 1988; Lalli and Gilmer, 1989) and seems generally inappropriate to describe their feeding strategy. Although no detailed studies exist to document the relative frequency of the various prey fractions, the external web (Gilmer, 1972, 1974; Gilmer and Harbison, 1986) provides an obvious trapping mechanism for fast swimming organisms. From mid-July to mid-August, 1988, metazoan zooplankton comprised an average of 45% by volume of items in the guts of 28 subarctic *Limacina helicina* (Phipps) preserved *in situ* (Gilmer and Harbison, unpub. data).

This paper describes observations, made with the use of scuba, of undisturbed thecosome individuals. These observations, some lasting up to 15 min, expand on previous observations (Gilmer, 1972; Gilmer and Harbison, 1986) and suggest ways that feeding webs could be produced, fished, and ingested. The term "mucous trapper" (Fallensteller), suggested by Richter (1977), is the most descriptive term relating to thecosome feeding behavior.

MATERIALS AND METHODS

Thecosome pteropods were observed and collected in hand-held glass jars by scuba divers during four cruises in the tropical and subtropical North Atlantic Ocean in May through August, 1986 (R/V "Oceanus" cruises 176, 177) in March, April, July and August 1987 (R/V "Oceanus" cruises 184, 191), in the arctic and subarctic Atlantic Ocean in July and August, 1988 (R/V "Endeavor" cruise, 182), and under sea ice in McMurdo Sound, Antarctica in November 1987. Individual thecosomes were observed and photographed for up to 15 min in the upper 30 m of the water column using standard blue water techniques (Hamner, 1975). To make feeding webs more visible, I used either carmine dye dispensed from a plastic squeeze bottle or strobe lighting. On night dives the absence of ambient light made webs easily visible with diving lights.

Photographs were taken with a Nikonos V underwater camera fitted with 1:1 or 1:2 close-up lenses and backlit from 10 to 30 cm with one or two Nikonos SB-103 underwater strobes. Either Kodak Panatomic X, Technical Pan black and white film, or Kodachrome 64 color films were used. Some individuals were photographed in successive intervals of 20 to 30 sec to record feeding sequences. Web diameters were estimated to the nearest 10 mm from field photographs. Shell dimensions were measured to the nearest 0.2 mm using a dissecting microscope and ocular micrometer. Activity of arctic *Limacina helicina* was measured by a diver swimming horizontally through dense populations and randomly counting whether individuals were motionless or swimming. Counts were made until 10 swimming individuals were observed.

RESULTS

LIMACINA HELICINA (LIMACINIDAE: EUTHECOSOMATA)

Limacina helicina retains its largest known size in subarctic Atlantic waters (Lalli and Gilmer, 1989). I measured feeding webs (Fig. 1a) up to 60 mm in diameter on specimens with 12.2 mm diameter shells (Table 1). *L. helicina antarctica* Woodward with shells measuring 4.6 mm in diameter (Table 1), fed from webs with estimated diameters of 20 mm. *L. helicina* is very sensitive to turbulence and usually abandons its feeding web in the presence of a diver. Careful placement of a camera framer around the animal can cause it to simply draw in the web. It is deflated like a balloon and appears to be drawn in dorsally between the wings near the large pallial opening (Fig. 1b). The web is withdrawn solely by ciliary actions in 15 to 30 sec.

Web production was not observed, but could be associated with a curious somersaulting behavior (Fig. 2) that is displayed by all of the euthecosome species I have observed. This behavior always takes place when swimming animals switch to a motionless, feeding posture (Fig. 1a). The animal slows its swimming speed, but at the same time increases wing motion and moves in a small arch roughly similar in dimension to its feeding web. At the apex, the animal moves in quick back and forth twists while continuing its path. Near the bottom of the arch when the shell is situated above the wings, the body quickly reserves position leaving the wings extended uppermost. Swimming motion stops immediately and the animal now hangs motionless in the water. The entire somersaulting sequence takes from eight to twelve sec to complete in all species that I have observed. *Limacina helicina* and *L. retroversa* (Fleming) sink slightly after somersaulting, but attain neutral buoyancy within 5 sec. This condition then lasts for at least eight min (the longest observation period), and is the only period when I have observed feeding webs in place. Neutral buoyancy, however, is also displayed by mating couples with no apparent aid from feeding webs or other mucus structures.

On cruise "Endeavor" 182 *Limacina helicina* occurred in surface waters in a distinct layer between 5 and 28 m. More than 97% of the individuals I surveyed ($n = 1200$) were neutrally buoyant and motionless in their feeding posture. This percentage was similar on all dives made between 0800 hr and 1900 hr at various stations over 27 days. Feeding individuals that were occasionally bumped by swimming *Limacina* showed no reaction to the contact and continued their quiescent posture. Several *Limacina* were even pulled a short distance in the water when the intruder became entangled in the web. Similar passive behavior by other feeding *L. helicina* was observed on four occasions when gammariid amphipods (> 5 mm body length) blundered into the pteropod web. The amphipods immediately broke free of the web. After their encounter with the amphipod, two of the *Limacina* swam off and the other two remained quiescent but did not appear to set new webs during two minutes of further observation.

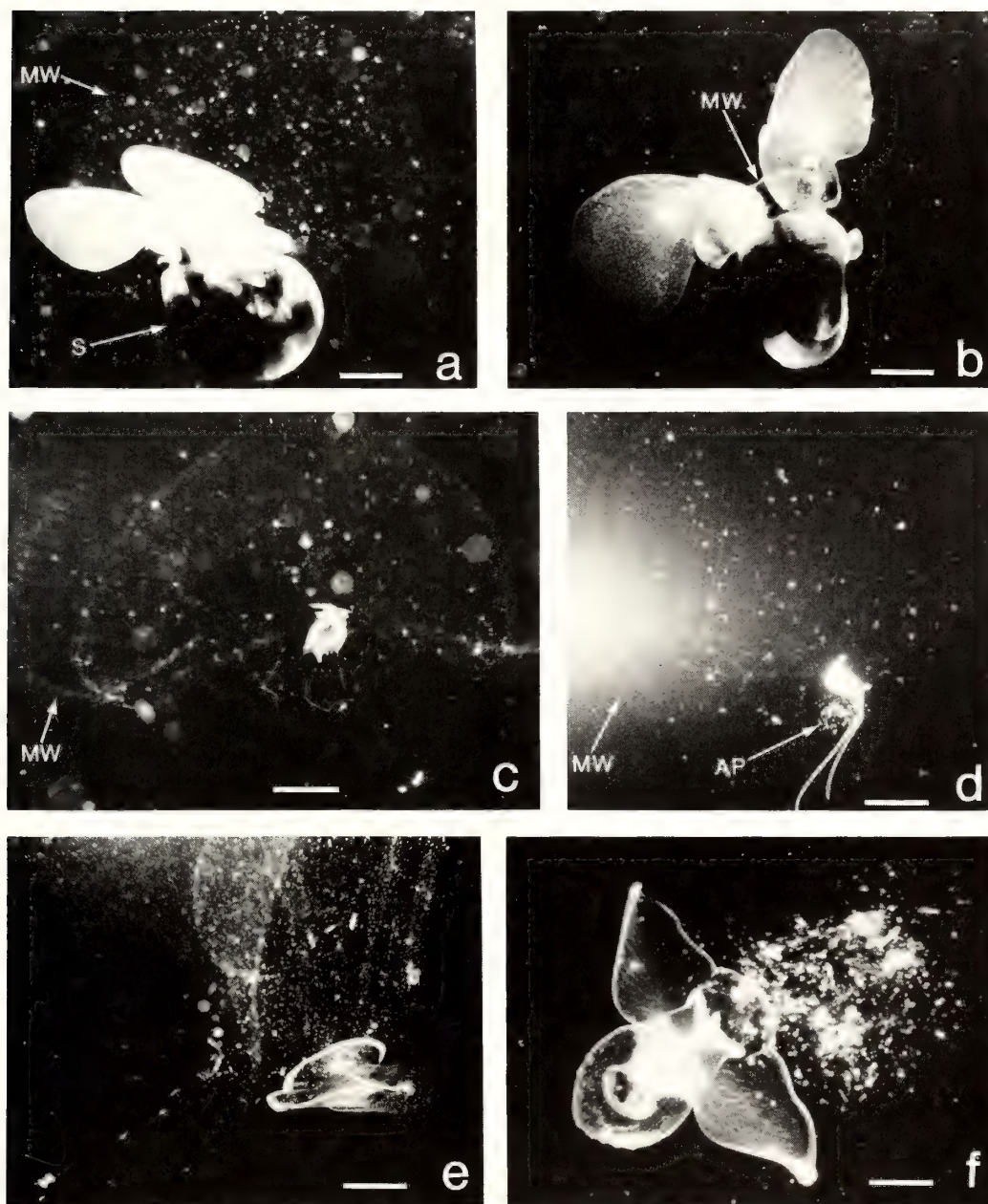


Fig. 1a. *Limacina helicina* in subarctic waters. Lateral view of individual in motionless feeding posture (S, shell; MW, mucous feeding web) (scale bar = 4 mm); **b.** *L. helicina*, dorsal view, in final stages of withdrawing feeding web (MW, mucous feeding web) (scale bar = 4 mm); **c.** *Cavolinia uncinata*, with extended mucous feeding web (MW) and no pseudofeces retained off the posterior shell margin (scale bar = 10 mm); **d.** *C. uncinata*, with extended feeding web (MW) and aggregated mass (AP) of pseudofeces and fecal pellets retained on the posterior shell margin indicating an earlier web was set in the same location (scale bar = 10 mm); **e.** *Corolla calceola* from northwestern Atlantic slope water with large mucous feeding web in place (scale bar = 25 mm); **f.** *C. calceola* with large mass of pseudofeces and feces after ingesting a feeding web (scale bar = 10 mm).

FAMILY CAVOLINIIDAE (EUTHECOSOMATA)

Within the upper 30 m, roughly 95% of the cavoliniids I observed maintained a quiescent feeding posture regardless of the time of day. The only exception to this is in turbulent mixing zones, such as langmuir cells or shear zones between warm and cold water masses (e.g. western edge of the Gulf

Stream and northwestern slope water interface), where our dive team has encountered vertical currents of approximately 0.5 knot. Here cavoliniids are often abundant and rapidly swim to maintain their position, or are swept away in the current. These are exceptional circumstances, as most thecosomes retain their motionless, feeding posture within 3 m of

the surface on rough days (e.g. Beaufort wind force 7).

Cavoliniids rapidly ingest their feeding webs in a manner similar to *Limacina helicina*. Webs are collected ventrally on the large expanse of the footlobes and funnelled into the mouth as condensed strings. At night, I have routinely observed *Clio pyramidata* Linné and *Cuvierina columnella* (Rang) withdraw their largest webs (Table 1) in 15 sec. Cavoliniids also display variable escape responses to the presence of a diver (Gilmer and Harbison, 1986). At one extreme of their behavior, individuals will flee from a small hand motion initiated up to 3 m away. Conversely, they will sometimes allow a diver to touch them several times before an escape response is induced. This sporadic escape behavior is characteristic of all thecosome species I have observed. Hand shading applied to change the illumination on a feeding individual elicits no escape response unless associated with turbulence.

I have observed *Cavolina uncinata* (Rang), *C. tridentata* (Niebuhr), *C. longirostris* (Blainville), *C. inflexa* (Lesueur) and *Diacria quadridentata* (Blainville) withdraw feeding webs, and then enter a non-fishing period that lasts for observed periods of up to 12 min. During this period, no web is present but pseudofeces and fecal pellets are transported down the dorsal surface and are retained posteriorly (Fig. 1d), as described previously (Gilmer and Harbison, 1986). Photographs of *C. uncinata*, *C. tridentata* and *D. quadridentata* suggest they occupy one position long enough to set, fish, and ingest at least two webs. Figure 1c shows a specimen of *C. uncinata* with a web in place, but with no fecal matter

or pseudofecal strings hanging from the posterior shell surface. Figure 1d shows another individual of this species with a web in place, but with fecal matter and pseudofecal strings present. This indicates a web was ingested and a new one set without swimming to a new location.

Particle laden webs of five *Cavolina tridentata* were observed during dives in northwestern Atlantic slope water. Four specimens left webs in place for five minutes, and one withdrew the web after three minutes, possibly because of diver turbulence. The latter specimen required almost one minute to withdraw its web. All individuals appeared to have produced webs in the same locations previously as fecal and pseudofecal material were present. These observations suggest *Cavolina* and *Diacria* can remain in one location for at least 35 min to set and fish sequential webs for five min each, and digest each web during 12 min "non-fishing" periods. Occasionally a combined mass of fecal pellets and pseudofeces can be identified *in situ* though I have never observed an intact abandoned web without having first disturbed an animal.

All cavoliniid genera display the same somersaulting behavior as described for *Limacina* (Fig. 2). I have also observed an extended pattern of this behavior by several specimens of *Creseis acicula* (Rang) and *Cavolina longirostris*. Initially, these individuals are motionless in feeding postures, but then sink away rapidly (approximately 5 cm/sec) for no apparent reason. After sinking 0.5 to 1.0 m, they somersault and again remain motionless. The somersault behavior in-

Table 1. Comparison of maximum dimensions of shells and feeding webs (in mm) of euthecosome species by region in the North Atlantic (includes data from Gilmer and Harbison, 1986).

Species	Location	Shell length ($\pm$ S.D.)	Max. Web Diameter	
<i>Limacina helicina</i>	subarctic Atlantic	12.2 (diameter)	60	(24/3)
	McMurdo Sound, Antarctica	4.6 (diameter)	20	(3/1)
<i>Cuvierina columnella</i>	north central Atlantic	11.0*	120	(13/5)
	slope water N.W. Atlantic	11.0*	110	(5/3)
<i>Clio pyramidata</i>	north central Atlantic	10.0 $\pm$ 0.4	50	(10/8)
	slope water N.W. Atlantic	10.0	40	(5/2)
<i>Cavolina longirostris</i>	north central Atlantic	5.4	40	(7/3)
	northern Sargasso Sea	5.0 $\pm$ 0.2	40	(5/1)
	Gulf Stream axis	5.6	50	(2/1)
	slope water N.W. Atlantic	5.8	40	(3/2)
<i>C. uncinata</i>	northern Sargasso Sea	7.0	100	(7/2)
	Gulf Stream axis	7.0	110	(9/5)
	slope water N.W. Atlantic	7.0 $\pm$ 0.2	110	(3/3)
<i>C. tridentata</i>	north central Atlantic	15.0	180	(2/1)
	northern Sargasso Sea	15.0	200	(1)
	Gulf Stream axis	15.4	200	(2/2)
	slope water N.W. Atlantic	15.0 $\pm$ 0.2	220	(6/4)
<i>Diacria quadridentata</i>	Gulf Stream axis	3.0	30	(2/2)
	slope water N.W. Atlantic	3.0	20	(2/1)
	Canary Current	3.6	20	(3/1)

() No. of webs measured/No. with maximum dimension

* excludes shell posterior to caudal septum

** visual estimation

volves the only swimming activity. I have observed several individuals each sink and somersault in this sequence up to four times in five min, with a net descent of approximately 3 m. At the end of this sequence the animal either remains motionless with no apparent web for the duration of the observation (up to five min), or swims off in a random direction out of the diving grid (> 20 m).

Small crustaceans often hover around feeding cavoliniids (Gilmer and Harbison, 1986), apparently attracted to the various surfaces as reported for larvacean mucous houses (Aldredge, 1972). During nighttime observations of *Cavolinia uncinata* (n=3), *Clio pyramidata* (n=15), and *Cuvierina columnella* (N=18), crustaceans (< 1 mm length) were usually observed inside the web as it was ingested. Initially, the crustaceans were free swimming inside the spherical web area, but some appeared to be captured in the mucous walls as the web was withdrawn. Four specimens I observed and collected at night each had an estimated 20 to 30 crustaceans trapped in their webs as they withdrew them, and all had crustaceans in their guts (Table 2). Only smaller crustaceans are successfully captured. Hyperiid amphipods (3 to 4 mm) attracted by the dive lights were often caught in the webs, but easily broke free after several rapid swimming motions.

Species occupying different water masses of the north Atlantic do not appear to alter their maximum web dimensions (Table 1). *Cavolinia tridentata*, *C. longirostris*, and *C. uncinata* were each observed with webs of similar dimension in central water masses of the temperate North Atlantic, the northern Sargasso Sea, the axis of the Gulf Stream, and in slope water along the northwestern Atlantic coast.

FAMILY CYMBULIIDAE (PSEUDOTHECOSOMATA)

The cymbuliids feed with enormous mucous webs that are often observed funnelled towards the footlobes as the animal lies below the web (Fig. 1e). Observations of *Corolla* and *Gleba* indicate that the web is slowly drawn in by the footlobes surrounding the mouth rather than the rapid, deflating balloon method observed with the spherical webs of euthecosomes. I have observed large numbers of *Corolla calceola* (Verrill) feeding in slope water regions of the northwest Atlantic. No apparent change in web size occurs during observations lasting up to 15 minutes. Food is ingested continuously and pseudofeces are released as long strands off the anterior side of the footlobes (see Lalli and Gilmer, 1989 for orientation in pseudothecosomes). I observed one specimen heavily laden with mucus and pseudofeces (Fig. 1f), suggesting that it had recently ingested a web. The mucus contained many copepods, larvaceans, and small diphyid siphonophores in addition to the waste matter. It actively swam twelve meters horizontally before freeing itself from the mucus, and then swam downwards out of our diving range (> 30 m). I have no observations to indicate whether cymbuliids ever set sequential webs in one location.

DISCUSSION

Among oceanic suspension feeders that employ

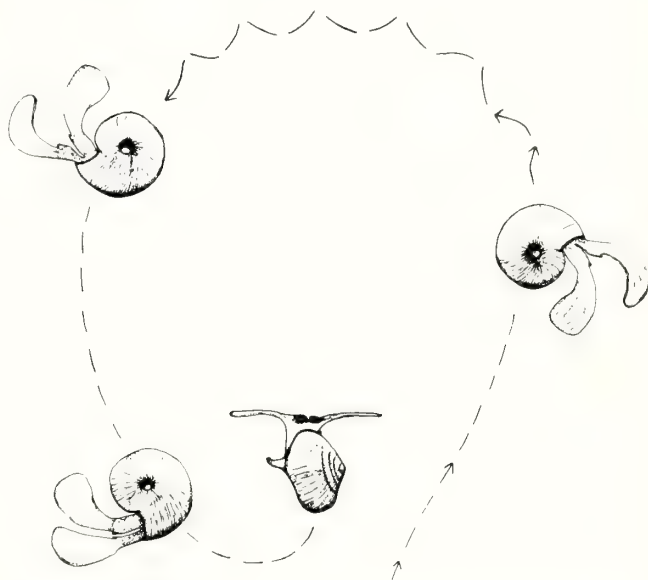


Fig. 2. Somersaulting behavior by *Limacina helicina* which occurs at the initiation of the motionless feeding posture. A similar behavior is displayed by all euthecosome genera.

mucous structures, thecosomes appear to feed by a novel trapping strategy. Although some larvaceans (Aldredge, 1976) and all doliolids (Diebel, 1982) share a motionless feeding posture with thecosomes, these tunicates pump water through their mucous filters and feed on very small particles. Salps feed in a manner more analogous to thecosomes, but move constantly with pumping motions and expose their feeding webs to continuous new water (Madin, 1974; Harbison and Gilmer, 1976). Whether thecosomes remain in one location or sink slightly during feeding, they use no active transport of water through the web as in tunicates. Thecosomes appear well suited to feeding by contract trapping of large motile organisms based on the occurrence of numerous crustaceans in and around the webs and in the gut contents of carefully collected specimens.

Although nighttime observations are the most limited in number, they have provided the most information about the trapping ability of the thecosome webs. All individuals I have closely observed feeding at night (n=35) capture numerous small crustaceans as they withdraw the web. The few specimens also collected during these dives have all had small copepods in their guts (Table 2). These observations remain qualitative, since much of the web material and potential food was undoubtedly lost during the capture. Intact crustaceans account for up to 25% by volume of intact items in guts of arctic *Limacina helicina* preserved *in situ* (Gilmer and Harbison, unpub. data). Copepods, however, are entirely absent in the laboratory for as little as three hours after capture. Small fragments of copepod exoskeleton are common in fecal pellets of *L. helicina*, especially segments of endopodites and thoraces.

The estimated times for *Cavolinia* and *Diacria* to fish two webs is limited by my short observation periods. The actual fishing times and the number of webs set in any one

Table 2. Gut contents of cavoliniids collected on night dives with crustaceans observed inside their feeding webs.

Species (Locality)	Dominant taxa	Max. dimension (μm)	No. of food items
<i>Cuvierina columnella</i> (n=2) (Northern Sargasso Sea)	copepod naupli	thorax < 600	5
	tintiniids	lorica 120	14
	thecate dinoflagellates	60	18
	<i>Globigerina</i> spp.	120	3
	centric diatoms	30	24
<i>Clip pyramidata</i> (N=1) (Northern Sargasso Sea)	copepod juvenile	thorax 1100	1
	copepod nauplii	thorax < 600	4
	tintiniids	lorica 110	21
	Radiolarians	150	4
	centric diatoms	30	4
<i>Cavolinia uncinata</i> (n=1) (Florida Current)	copepod nauplii	thorax < 700	3
	<i>Limacina inflata</i> juv.	shell < 300	2
	tintinnids	lorica 140	6
	thecate dinoflagellates	90	7

location are undoubtedly much greater, judging from the extensive amount of pseudofeces that some individuals accumulate. This quiescent behavior may explain how well developed hydroid colonies exist on the shells of some thecosomes. *Kinetocodium danae* Kramp often found on *Diacria trispinosa* (Blainville) (Lalli and Gilmer, 1989) has feeding polyps that could easily reach the web surface to prey upon attracted crustaceans. Hydroids I have observed on other thecosomes have feeding polyps either situated near the anterior portion of the shell, nearest the host feeding web, or have stalked feeding polyps that could reach the host web from other regions of attachment on the shell.

The large, sheet-like webs used by pseudothecosomes and the slow methodical fashion of ingesting them is easily comparable to the feeding style of the vermetid prosobranchs (Hughes and Lewis, 1974; Hughes, 1978). In contrast, the spherical webs used by euthecosomes and their ability to rapidly ingest them appears to be unique among marine animals that feed with mucous structures.

Much of the feeding behavior of thecosomes remains obscure. For instance, webs produced by *Limacina* appear to arrest sinking and provide neutral buoyancy during feeding, although mating couples display neutral buoyancy as well and have no feeding webs in place. Secondly, the somersaulting action displayed by all euthecosomes does not appear to coincide with the setting of a web, but always occurs prior to initiation of the feeding posture. Finally, it is unclear how free swimming copepods penetrate the walls of the euthecosome webs without adhering to them. Hopefully, future *in situ* observations and collections will help to answer these questions and may ultimately help determine the precise, thecosome feeding strategy.

ACKNOWLEDGMENTS

I wish to thank Dr. G. R. Harbison for financial support and for allowing me time to make these observations during cruises. I also thank G. R. Dietzmann for valuable assistance during night dives.

Research support was provided by N.S.F. grants OCE 85-16083, OCE 87-46136, and DPP 86-13388 to G. R. Harbison. This is Harbor Branch Oceanographic Institution Contribution No. 748.

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- Date of manuscript acceptance: 25 November 1989

MATING BEHAVIOR AND SPAWNING IN TWO NEUSTONIC NUDIBRANCHS IN THE FAMILY GLAUCIDAE

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ABSTRACT

We observed the mating behavior and egg production rates of *Glaucus atlanticus* (Forster, 1777) and *Glaucilla marginata* (Bergh, 1868), neustonic nudibranchs of the family Glaucidae, collected from the western Pacific Ocean near Australia. Although the same sequence of mating behaviors occurred in both species, the timing of these behaviors and mating duration were different. The entire mating sequence from "kiss" to separation lasted about one hour in *G. atlanticus* and about one minute in *G. marginata*. Morphological differences that could be associated with this difference are discussed. Glaucids release short gelatinous strings of eggs at varying frequencies. Egg production rates in both species were directly related to food availability, though both species continued to produce eggs at lower rates for several days with little or no food. Both glaucids exhibited several characteristics of planktotrophic development: eggs were small (70 μm by 80 μm), embryonic developmental times were short (2.5 to 3.0 days at 19°C), and veligers swam longer than a week.

Only a small number of nudibranchs spend their entire life in the water column, and these species have few evolutionary adaptations to pelagic life. Several characteristics, such as no heavy shell and some swimming ability, that could be considered advantages in a pelagic habitat are also found in benthic nudibranchs. Some pelagic nudibranchs are flattened or otherwise modified and do not resemble benthic nudibranchs, but two species of Glaucidae, *Glaucus atlanticus* (Forster, 1777) and *Glaucilla marginata* (Bergh, 1868), are easily identified as eolid nudibranchs by their cerata. Like other nudibranchs, the Glaucidae are reciprocal hermaphrodites, although little is known about their mating behavior and spawning characteristics (Lalli and Gilmer, 1989).

Both glaucids are neustonic, occurring in the surface layer of tropical oceans. *Glaucus atlanticus* is circumtropical. *Glaucilla marginata* is restricted to the tropical Pacific Ocean. Both species float upside down on the air-seawater interface and neither is a good swimmer. Their distribution is primarily controlled by winds, as is also true of their cnidarian prey, *Physalia*, *Veella*, and *Porpita* (Lalli and Gilmer, 1989). Like other neustonic animals, they are countershaded, blue to purple on their ventral surface which faces up, and white or silvery on their dorsal side facing down. Thompson and Bennett (1970) discovered that *G. atlanticus* stores nematocysts derived from their prey in cnidosacs at the tips of the cerata. The

utilization of nematocysts as a defense against predators is well known in eolid nudibranchs, although the processes involved in the control of the discharge of these nematocysts are not well understood (Todd, 1981; Thompson and Bennett, 1970).

There are several differences between *Glaucus atlanticus* and *Glaucilla marginata* that are important to a discussion of their mating behavior (Lalli and Gilmer, 1989). *G. atlanticus* is the larger of the two species, reaching a maximum reported length of 43 mm (Miller, 1974). It has a long slender foot, and a long, strong, contractile penis armed with a chitinous spine. The long cerata are arranged in single rows in three to four clusters projecting from lobes on the sides of the body. The central ceras is much longer than those on the sides of the cluster. A single individual can have up to 85 cerata. *G. marginata* is smaller than *G. atlanticus*, with previous recorded sizes up to 12 mm, and up to 18 mm in this study. The penis is not armed and the cerata are arranged in multiple layers in four clusters. *G. marginata* can have twice as many cerata as *G. atlanticus*. In both species the reproductive aperture is just posterior and level with the bottom of the first right ceratal arch.

Despite their widespread distribution, observations and experiments on live *Glaucus atlanticus* and *Glaucilla marginata* are rare, perhaps because they are usually damaged when

when they are collected with nets. In this paper we will compare the pattern and timing of mating behavior in these two pelagic nudibranchs. We will also describe and quantify spawning and egg production in both species, and the effect of starvation on egg production in *G. marginata*. Some observations of the egg ribbons and embryonic developmental times will be described. However, veligers did not complete metamorphosis to adult in our laboratory.

METHODOLOGY

COLLECTION AND MAINTENANCE OF ANIMALS

We found glaucid nudibranchs in the surf zone and blown onto the beaches of New South Wales, Australia, south of Sydney, in the austral summer of 1979-1980. *Glaucus atlanticus* were collected in early December from Morulga and Bawley Beaches, and *Glaucus marginata* were collected in early December from North Kioloa Beach, and early March from South Kioloa Beach. No glaucids were found on a search of the sand and surf zones of a group of beaches in this area from mid-December until late February. The beaches were regularly inspected by the manager of the Kioloa Field Station of the Australian National University (ANU). Surf zone temperatures were 20.5°C on 11-12 December, and 22.0°C on 2 March. We scooped glaucids from the surf into containers of seawater and returned them to the laboratory at ANU.

At ANU, adult glaucids were kept in three 10.0 l rectangular seawater aquaria (two per aquarium) at room temperature (about 20°C) or at 19°C in shallow 1.0 l plastic boxes. Seawater in the containers was changed two to three times a week. Both glaucids were fed either fish food or pieces of *Physalia* sp. collected from the same beaches and frozen until use.

MATING BEHAVIOR

We observed the sequence and timing of mating behavior for three pairs of *Glaucus atlanticus* and six pairs of *Glaucilla marginata*. Mating behavior was observed and timed either in the aquaria or in large shallow containers.

SPAWNING BEHAVIOR AND EGG PRODUCTION RATES

Glaucids release strings of eggs, and do not lay down an egg mass like other eolid nudibranchs. Dimensions of egg capsules from seven strings released by *Glaucus atlanticus* were measured using a compound microscope with a calibrated micrometer. In addition, we measured the lengths of 20 strings and the number of eggs in each string for strings released four days after collection. Time intervals between release of strings were recorded for five individuals in order to calculate daily fecundities. Fresh spermatozoa were also measured using a compound microscope. For *Glaucilla marginata*, egg capsule size was measured in 11 strings released by adults collected in March.

Ten *Glaucilla marginata* collected in March were used to evaluate the response of egg production rate to a decrease in food availability. Each adult was isolated immediately after

collection, and removed from the jar and placed in fresh seawater at 12, 24 and 36 hr after collection. All the strings and the number of embryos per string in at least 15 strings were counted for each individual for each of the three 12 hr periods. The length of the string was also measured for the first 12 hr period. At the end of 36 hr, the total length and wet weight in grams of each individual were measured. We estimated the egg production rate for freshly collected animals and for animals kept without food for periods up to 36 hr.

DEVELOPMENT

Some egg strings were maintained in aerated glass jars at 19°C to determine embryonic developmental times, and to observe survival and behavior of the larvae after hatching. The age of the strings was known to within 12 hr. Embryos of both glaucids hatched. Veligers were inspected for continued survival and swimming ability until they sank to the bottom of the jars and died.

RESULTS

MATING BEHAVIOR

Mating in both *Glaucus atlanticus* and *Glaucilla marginata* involved a sequence of predictable and stereotypic behaviors. These behaviors were the same for both species and began when conspecific glaucids contacted each other. Although there was some variation in the exact timing of each behavior type in the sequence, total duration of mating was similar for all pairs of the same species.

The first in the sequence of behaviors was the relatively brief "kiss". Partners oriented so that their mouths and ventral surfaces were touching, and the heads usually submerged. Shortly after the mouths joined, the penises emerged. The penises were greatly extended, and sometimes the end of one penis was wrapped around the other individual. The second major behavior was the intertwining of the two penises, and the two individuals began to couple. Initially the mouths separated, but the pair were still oriented ventral surface to ventral surface (Fig. 1a). Both glaucids arched the body and the cerata clusters toward their dorsal surfaces, keeping the cerata away from each other. During mating we observed individuals flinch and arch away when touched by the cerata of the mate. This observation indicated that a ceras could be stimulated by this contact to eject the cnidophages in the cnidosacs at the tip of the ceras, and the partner could be stung by the nematocysts from the cnidophage. Shortly after coupling began, the pair changed to a side by side orientation with the ventral surfaces on the air-water interface and the penises lying between the two parallel bodies (Fig. 1b). During coupling the pair could be either head to head or head to tail, and often switched from one to the other once or twice during this time period. The pair lay quietly between changes in orientation. Penises were loosely intertwined until two-thirds through the coupling period when the penises became tightly intertwined (Fig. 1c). When the pair began to separate, the coil untwisted, the penises showed some thickening as they started to retract, and the nudibranchs flexed their bodies (Fig. 1d). Total retraction of the penises required a much longer



Fig. 1. *Glaucus atlanticus*. Sequence of mating behaviors: (a) 7 min after initial contact, ventral surface to ventral surface; (b) 37 min after initial contact, coupled, penises in loose coil, ventral surfaces at the air/water interface; (c) 47 min after initial contact, coupled, penises in tight coil; (d) 58 min after initial contact, separated, penises beginning to retract, note thickening. Horizontal field width = 40 mm.

period of time than separation of the two individuals.

Although the pattern of behavior for the two species of glaucids was identical, the timing of each behavior and the duration of mating was very different. For *Glaucus atlanticus*, the "kiss" lasted about four min. Between four and nine min into the sequence the pair reoriented so they were parallel, usually head to tail, with the penises loosely coiled between them. The coil tightened 36 to 41 min into the sequence, remained tight for four to nine min, then loosened again. Separation took four to nine min and was complete 43 to 59 min after the initial joining. Retraction of the penis took up to an hour (19, 52 and 61 min).

For *Glaucilla marginata*, the mating sequence was substantially shorter. The "kiss" lasted five sec. Pairs then twisted, and oriented parallel to each other with their ventral surfaces facing up and penises twisted around each other. Coupling lasted 60 sec (range 50 to 70 sec, SD = 8.4 sec). Separation was fast, and the penises retracted quickly, within 21 sec (range 10 to 35 sec, SD = 8.6 sec). The total time for mating was 65 sec, about 2% of the time taken by *Glaucus atlanticus*.

The ability to mate again with the same or a different individual immediately after mating was investigated in both species. A pair did not remate after retraction of the penises, even if they were pushed together. But we observed that a

third non-mated glaucid will extrude its penis in the presence of a mating pair. In *Glaucilla marginata* we saw these new individuals mating with recently mated glaucids.

SPAWNING AND EGG PRODUCTION

Both species released their eggs in straight strings about 0.3 mm in diameter that sank slowly (about 50 m d⁻¹). Strings were from 5.0 to 17.5 mm long for *Glaucus atlanticus*, and from 2.0 to 6.4 mm for *Glaucilla marginata*. In both species ova were individually encapsulated (primary membrane). Egg capsules were oval, and evenly spaced slightly less than one diameter apart within the egg string. A thin transparent tube (secondary membrane) surrounded the egg capsules within the mucous string (Fig. 2). Egg capsules of the two glaucids were similar in size: *G. atlanticus*, 60 to 75 μm wide and 75 to 97 μm long; and *G. marginata*, 58 to 67 μm wide and 74 to 82 μm long. Sperm of *G. atlanticus* were long and slender (129 μm by 0.9 μm).

Daily or hourly fecundity was a function of the number of embryos in a string, and the rate of string production. The number of eggs per string was a linear function of the length of the string, but smaller *Glaucilla marginata* released strings that were less than half the length and contained less than half the embryos of those released by *Glaucus atlanticus* (Fig. 3). The time interval between strings was also different for

the two species. Even after three to four days in the laboratory *G. atlanticus* produced 4-6 strings/hr (average for five individuals was 3.8 strings/hr). *G. marginata* released 8.6 strings/hr during the first 12 hr after collection, twice the frequency of *G. atlanticus*. However, the frequency dropped to less than two strings an hour during the next 12 hr. Since the number of embryos per string ranged from 36 to 96, *G. atlanticus* released from 3300 to 8900 embryos/day, even after three to four days in the laboratory. The fecundity for freshly collected *G. atlanticus* was about the same, from 1850 to 9250 embryos/day.

The relationship between size and egg production and the effect of starvation on egg production was quantified for *Glaucilla marginata*. First, although total length (TL) and wet weight of the ten individuals measured were significantly correlated, egg production (EP in number/hr) was more closely related to total length ($EP = 597.2 TL - 571.2$, $r^2 = 0.56$) (Fig. 4) than to wet weight ($r^2 = 0.27$). The increase in egg production with increasing size was a function of both increased string production (strings/hr, $r_s = 0.705$) and closer packing of egg capsules in a string (eggs per string, $r_s = 0.675$), but not an increase in the length of the string with total length of the glaucid ($r_s = 0.421$, ns) (Spearman's rank correlation coefficient, $p = 0.05$; Siegel, 1956). The total length of *G. marginata* ranged from 12.6 to 17.9 mm, string production ranged from 3.3 to 12.9 strings/hr, and the average number of eggs per string ranged from 25.5 to 41.7 ($n = 15$ strings per individual). The average string length of the same 15 strings ranged from 3.8 to 5.1 mm, with 95% confidence intervals of 0.2 to 0.5 mm. Differences in average string length between individuals was not a significant factor in the increase in egg production with size.

Egg production rates of starved *Glaucilla marginata* decreased significantly after 12 hours ($X^2_2 = 15.2$, Friedman two-way ANOVA, $p > 0.001$; Siegel, 1956), but then remained the same during the remainder of the experiment (Fig. 5). Egg production rates in the first 12 hr after collection were 3.5 times rates in the second and third 12 hr intervals after collection,

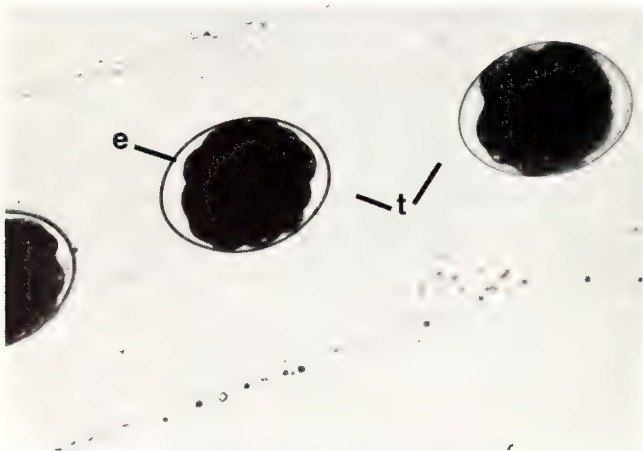


Fig. 2. *Glaucus atlanticus*. Development at 19°C; embryos about 24 hours after release, multiple cell stage [e, egg capsule (primary membrane); t, internal tube (secondary membrane)]. Horizontal field width = 300 μ m.

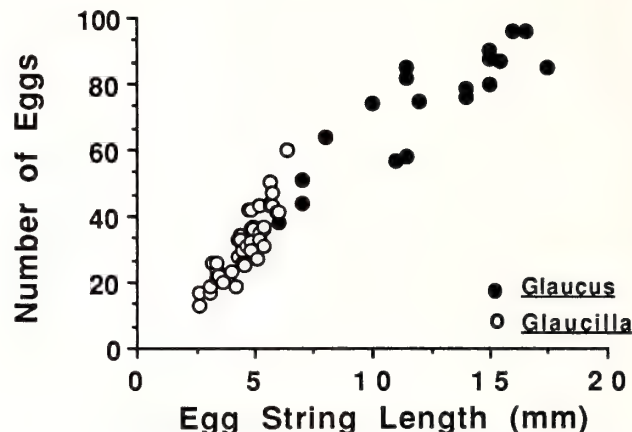


Fig. 3. Relationship between egg string length and the number of eggs per string for *Glaucus atlanticus* (• = one pair) and *Glaucilla marginata* (o = 4 individuals).

which were the same ($U = 43$, Mann Whitney U, $p = 0.05$, Siegel, 1956). *G. marginata* was still producing eggs after 36 hr with no food.

Lower egg production rates in starved *Glaucilla marginata* were due to fewer strings produced/hr and fewer egg capsules per string. String production decreased to 36 to 39% of initial rates, and egg capsules per string to 59 to 71% of initial values. Although the decrease in egg capsules per string could be due to shorter strings or to greater spacing between the egg capsules or both, four of the ten *G. marginata* produced some very short strings with only one or two eggs per string during the second and third 12 hr periods of starvation. Thus string length could decrease during starvation.

DEVELOPMENT

Embryos of *Glaucus atlanticus* began to divide after a few hours at 19°C. At about 24 hr, the embryos were multicellular. The egg capsules were still separated within the string, but the secondary membrane was thinner and constricted between the embryos (Fig. 2). Between 48 and 60 hr, embryos had beating cilia (trochophore), and the secondary membrane began to disintegrate. After about three days at 19°C, the half-shelled veligers moved slowly through the mucous string and swam away. The shell was initially ovoid but became coiled after a few days. Starved veligers of *G. atlanticus* swam continuously for seven to 11 days after hatching before sinking to the bottom of the containers, and dying before metamorphosis into juveniles. The shell was 89 μ m by 104 μ m, larger than the egg capsule. Embryonic developmental time for *Glaucilla marginata* was about the same, 2.5 to 3.0 days at 19°C. These embryonic developmental times were slightly slower than found for *G. atlanticus* veligers maintained at 25°C (2.0 days, Bebbington, 1986). The bilobed veligers of *G. marginata* swam continuously for 33 days after hatching before they died without metamorphosing. The shell was 96 μ m by 119 μ m.

Thus both species showed characteristics of planktonic development: small eggs, short embryonic

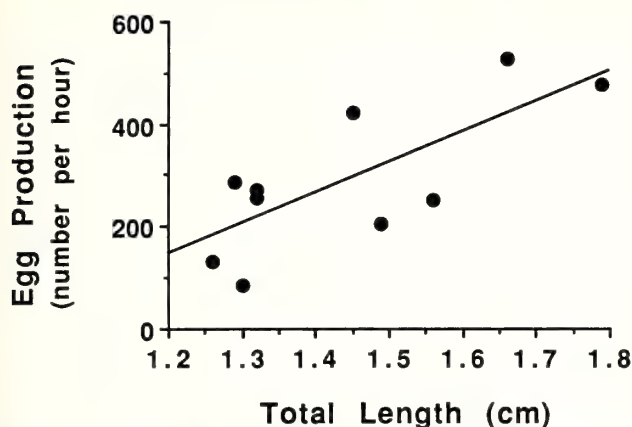


Fig. 4. *Glaucilla marginata*. Relationship between animal size, total length, and egg production 12 hr after collection.

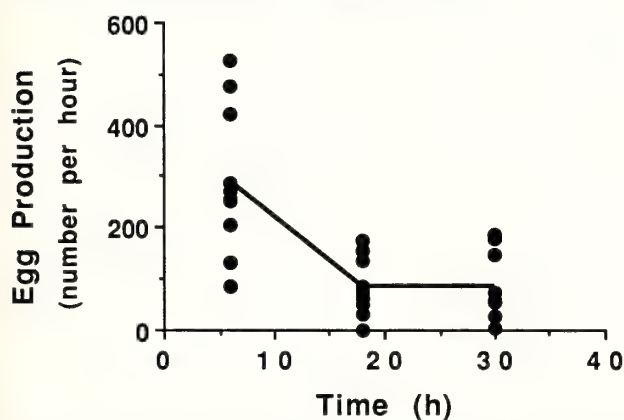


Fig. 5. *Glaucilla marginata*. Decrease in egg production over time with no food available. Points are average egg production rates for three 12 hr intervals after collection for 10 individuals.

developmental times, and veligers that spend several weeks swimming in the plankton. Veligers of *Glaucus marginata* survived three times longer in the laboratory than did veligers of *Glaucus atlanticus*, but the veligers of *G. marginata* were given a mixture of marine phytoplankton which could have increased their survival times. We were unable to stimulate metamorphosis, but are unsure whether the inability of the veligers to metamorphose was due to inadequate diets or lack of the appropriate substrate as is necessary for most eolid nudibranchs (Harrigan and Alkon, 1978; Thompson and Brown, 1984).

DISCUSSION

Glaucus atlanticus found on the beaches of New South Wales in Australia were of the typical color pattern and within the size range found elsewhere in the world (Bennett, 1836; Bieri, 1966; Miller, 1974; Thompson and McFarlane, 1967). *Glaucilla marginata* have been recorded only once before from Australian waters (Thompson and Bennett, 1970). *G. marginata* collected in this study were 18 mm in length, much larger than previously reported (Thompson and Bennett, 1970), and the ventral surface was not brown but deep purple - similar to *G.*

atlanticus. Differences in coloration could be due to differences in diet between the two groups of *G. marginata*, as found for other pelagic nudibranchs (Lalli and Gilmer, 1989). We never found the two species of glaucids together, but both species were always found with some of their cnidarian prey.

Previous reports of mating behavior are sparse for *Glaucus atlanticus* and nonexistent for *Glaucilla marginata* (Lalli and Gilmer, 1989). Possibly because he did not observe the complete stereotypic mating sequence of *G. atlanticus*, Bebbington (1986) stated that *G. atlanticus* paired laterally or ventrally during copulation in the laboratory. We found that *G. atlanticus* pairs both laterally and ventrally, but at different times during the same mating sequence.

Mating in *Glaucus atlanticus* was exceptionally long compared to other pelagic nudibranchs, whereas mating duration in *Glaucilla marginata* was similar to other species of pelagic nudibranchs, one to fifteen minutes (Lalli and Gilmer, 1989). Maximizing the reproductive potential of each encounter may be particularly important to a pelagic species that must depend on chance encounters to find a mate. In the pelagic realm there are several options for exchanging large amounts of sperm: an exchange of spermatophores as found in heteropods and thecosomes; prolonged mating as found in gymnosomes and *G. atlanticus*; mating with many partners sequentially in swarms or rafts as is true of one species of pelagic dendronotacean (Lalli and Gilmer, 1988).

For the neustonic glaucids, wave and wind action at the surface make prolonged mating difficult. First, wave action will tend to separate partners. Second, both glaucids utilize nematocysts (Lalli and Gilmer, 1989). Contact with a mate could stimulate the contraction of the muscle complex around the cnidosac and the release of the cnidophage and nematocysts into the water, thus stinging the partner. In eolid nudibranchs, the nematocysts are in cnidophage cells inside the cnidosac. When the muscles surrounding the cnidosac contract, the cnidophage is ejected through the cnidopore or the epithelium at the tip of the ceras. If the cnidophage membrane ruptures on release, the nematocysts usually discharge (Greenwood and Mariscal, 1984). Stimulation of special neurosensory cilia which are concentrated at the ceras tip could cause contraction of the cnidosac wall (Todd, 1981). Mere pinching of the ceras with metal forceps (Thompson, 1976) or pressure on a cover slip (Greenwood and Mariscal, 1984) will stimulate ejection of cnidophage cells in many eolids. Both glaucid species actively avoid each other's cerata, and individuals flinch when touched by the cerata of their partner during mating. For benthic eolid nudibranchs neither problem occurs. Wave action is minimal, and the cerata tips are oriented dorsally not to the side as is true of the glaucids.

Morphological adaptations in *Glaucus atlanticus* appear to have solved both of these difficulties. The chitinous spine on the penis of *G. atlanticus* may help prolong contact in the face of wave action, and thus may be a singularly important morphological adaptation to long mating times (Miller, 1974). In contrast, pelagic nudibranchs with shorter mating times have unarmed penises. In addition, in *G. atlanticus* the cerata are fewer, longer and in a single layer, so are more easily held away from the partner than in *Glaucilla marginata*.

Prolonged copulation may mean more sperm are exchanged, filling the seminal receptacle. If prey are available, fertile egg production can continue longer before finding another mate is necessary. This could be advantageous when finding a mate depends primarily on physical forces in the ocean and not active searching. With the exception of *G. marginata*, the other species of pelagic nudibranchs are more active swimmers, and/or mate in swarms or cling to a surface flotsam (Lalli and Gilmer, 1989), so finding a mate is not as dependent upon physical forces in the ocean.

Our observations of the frequency of egg string production and of the number of eggs per string for *Glaucus atlanticus* were different from those of Bebbington (1986) and Macnae (1954). Although Bebbington saw fewer ova per string, strings were produced much more frequently, leading to fecundity estimates about six times ours. Bebbington gives no information about how long his glaucids were kept in the laboratory or their feeding conditions. Macnae (1954) also found fewer ova per string and greater spacing between embryos than we did, but did not estimate frequency of string production. We observed that egg production in *G. atlanticus* increased within hours of ingesting a slurry of homogenized *Physalia*. There was no interval between strings, instead of the 10 to 15 min interval between strings found when this species was fed fish food. These combined observations on egg production in *G. atlanticus* in conjunction with the results of the experiment on the effect of starvation on egg production in *Glaucilla marginata* suggest that egg production in glaucids may be closely coupled to their recent feeding history. Thus the inter- and intraspecific differences in rates of egg production observed for the two glaucids may be due to differences in their immediate nutritional histories.

There appear to be no major adaptive differences in reproduction and development between these neustonic eolids and their benthic relatives. Egg strings have the same basic form (hollow, cylindrical, capsule-filled cord) as benthic eolids, but the ribbon floats free as a short uncoiled string of eggs such as found in other pelagic nudibranchs instead of being attached on one side (Lalli and Gilmer, 1989). Many other species have only one ova per capsule (Hurst, 1967). The presence of a secondary membrane is not common, but its function is unknown (Eyster, 1986). Glaucids have relatively high fecundities compared to benthic eolid nudibranchs of the same size range (Harris, 1975; Rivest, 1978) and compared to the only other pelagic nudibranch, *Phylliroe bucephala* (Peron and Lesueur, 1810), for which we have fecundity estimates (Lalli and Gilmer, 1989). High fecundities and a direct linkage of egg production to food availability are valuable characteristics for these neustonic glaucids which have an unpredictable food source that occurs in large quantities.

ACKNOWLEDGMENTS

This research was conducted while L. B. Quetin was a Queen Elizabeth II Fellow in Marine Biology and R. M. Ross was a Visiting Fellow in the Research School of Biological Sciences at the Australian National University. We would like to thank Dr. E. E. Ball and the staff and scientists in RSBS and at the Kioloa Field Station for their sup-

port and for the use of the facilities. Comments from two anonymous reviewers and a discussion with L. Harris helped clarify our thoughts.

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Date of manuscript acceptance: 25 March 1990

BIPOLAR VARIATION IN *CLIONE*, A GYMNASOMATOUS PTEROPOD

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ABSTRACT

The gymnosome *Clione* inhabits waters of the Arctic, North Pacific, North Atlantic, South Atlantic and Antarctic Oceans. This study reexamines bipolarity in this genus, utilizing new morphological techniques as well as observations of living animals in all locales. Differences in external anatomy are readily apparent in both larval and adult *Clione* from northern and southern areas, and scanning electron microscopy has revealed differences in the number and size of hooks in animals from different regions. The configuration of the radula differs between specimens from the northern and southern hemispheres, and *Clione* from the Antarctic lacks median radular teeth, in contrast to the well developed, sickle-shaped median teeth present in *Clione* from northern areas. *Clione* acts as a food specialist throughout its range, feeding either on the bipolar thecosome species *Limacina helicina* (Phipps) in cold waters or *L. retroversa* (Fleming) in temperate waters. Differences in maximum adult size of *Clione* can be related to the size of available prey. Although there do not appear to be differences in the feeding behavior of *Clione* from different areas, there are slight differences in swimming and escape behavior, particularly between specimens of *Clione* from northern and southern regions. The results of these anatomical and behavioral observations support the validity of recognizing two species of *Clione*, *C. limacina* (Phipps) in the northern hemisphere and *C. antarctica* (Smith) in the Antarctic and South Atlantic oceans.

The gymnosome now called *Clione limacina* (Phipps) was originally illustrated in a publication by Martens in 1675, from specimens collected in waters off Spitzbergen and Greenland. However, Captain C. J. Phipps is credited with the first taxonomic description, which appeared in his 1774 book entitled *A Voyage Towards the North Pole*. Since these early descriptions, many publications have dealt with this species, the most abundant gymnosome in temperate and cold northern waters. It has been recognized as an important food for baleen whales and certain commercial species of fish (see Lalli and Gilmer, 1989).

Since the late 1700s, a species of *Clione* has also been known to exist in cold waters of the southern hemisphere. Early illustrations and taxonomic history of the northern and southern *Clione* can be found in Rang and Souleyet (1852) under the respective names of *Clio borealis* (Rang and Souleyet) and *C. australis* (Rang and Souleyet). The southern

Clione has been variously considered as a variety, form, or subspecies of *C. limacina* [see van der Spoel (1976) for synonymy], or as a separate species, *C. antarctica*, described by Smith in 1902. The southern *Clione* is less well known than the Arctic/Subarctic species, but preserved specimens were admirably described by Eliot (1907), who pointed out several anatomical differences between *C. antarctica* (Smith) and northern *C. limacina*. Additional anatomical studies of *C. antarctica* have been made by Meisenheimer (1906), Massy (1920, 1932) and Pruvot-Fol (1932), among others.

Clione limacina is found throughout the central Arctic Ocean, and extends in Subarctic waters southward into both the North Atlantic and North Pacific to approximately 30° to 40° North latitude. The southern hemisphere *Clione* is found circumglobally in Subantarctic and Antarctic waters, extending northward to about 40°. Because there are no connecting populations in tropical areas, these gymnosomes have

been considered as one of several examples of bipolarity in plankton (Ekman, 1953).

Our objectives here included direct comparisons of the morphological and behavioral differences between northern and southern populations of *Clione* in order to investigate their taxonomic distinction. We have compared our behavioral observations of several hundred living animals made during the Hudson 70 Expedition to Antarctic waters in 1970 by one of us (CML) and during two visits to Antarctica in 1987 and 1988 by the other of us (RWG), to those of living *Clione* observed in both the North Atlantic and North Pacific over a period of many years. We have also made anatomical and histological comparisons of specimens of *Clione* from several locations, using scanning electron microscopy to investigate detail not noted previously.

METHODS

Specimens of *Clione* were collected by plankton nets or by scuba divers at various locations throughout its range. Behavioral observations were noted *in situ* by divers or in laboratory aquaria. For anatomical studies, specimens representing a range of size categories were narcotized with MS222 (Ethyl m-aminobenzoate) and then preserved in either Bouin's fluid or 4% formaldehyde. Radulae and hooks were dissected from specimens, and extraneous tissue was removed by soaking in a dilute bleach solution. After rinsing in an alcohol series, the buccal structures were mounted on stubs with double stick tape, coated with gold, and examined on either a Cambridge Stereoscan 250 or a Novascan 30 scanning electron microscope. In all, radulae were examined from over 20 specimens collected across the regions under study. Histological sections of 10 μ m thickness were made of four *Clione antarctica* and stained using Cason's modification of Mallory's triple stain (Humason, 1962).

RESULTS

COMPARISON OF EXTERNAL ADULT MORPHOLOGY

Adult specimens of *Clione limacina* from the North Pacific and North Atlantic are anatomically similar (Figs. 1a, b), despite differences in size. *Clione* attains a maximal size of about 30 mm in length in the North Pacific Ocean, whereas adult specimens in arctic and subarctic waters of the North Atlantic Ocean commonly reach a length of 70 to 85 mm (Table 1), the largest size of any gymnosome. The maximal size of *C. limacina* diminishes in more temperate waters of the North Atlantic Ocean; sexually mature specimens are usually less than 25 mm long in waters south of Nova Scotia, Canada, and are less than 12 mm long in the English Channel.

Clione antarctica (Fig. 1c) attain a maximal length of about 42 mm, and it is strikingly different in external appearance from the northern species of *Clione*. The head of *C. antarctica* is elongate compared with that of *C. limacina*, and the demarkation between the head and the remainder of the body is not as clear. The head comprises about one-quarter to one-fifth of the total body length, whereas the head

of *C. limacina* is smaller relative to total body length, constituting less than one-seventh of the total body length of extended, living animals. The anterior tentacles of *C. antarctica* are noticeably smaller than those of the northern hemisphere species, but both species have similarly sized posterior tentacles located in depressions on the dorsal surface of the head.

Clione antarctica adults retain evidence of all three larval ciliary rings that are characteristic of gymnosome polytroch larvae, although the cilia can disappear. The first ring persists as distinct and separate protuberances encircling the mid-section of the head. The second and third rings persist as transparent bands that encircle the mid-trunk and the posterior tip of the body, respectively. In *C. limacina*, the anterior and middle larval rings disappear rapidly and completely during metamorphosis of the polytrochous larva to the juvenile form; the third or posterior band can persist through the juvenile stage, but it is not evident in sexually mature adults, except in English Channel populations. The posterior tip of the body of adult *C. antarctica* is marked by the constriction of the posterior larval ring, followed by an expanded triangular area. This is identical to the form of the posterior tip in young juvenile *C. limacina* but, in adults, the constriction is lost and the posterior end tapers gradually to a point.

Adults of *Clione antarctica* also differ from the northern species in that the viscera extends three-quarters of the way to the posterior tip of the body. In *C. limacina*, the viscera can occupy the entire body of larvae and juveniles but, in adults, the visceral mass does not extend beyond the anterior one-half of the body; the posterior half of the adult body is a fluid-filled cavity.

Body coloration is somewhat variable among individuals but, in both species, the prehensile buccal cones are reddish-orange. The posterior tip is brightly pigmented in some specimens of *Clione limacina*, but it is usually transparent or only faintly colored in *C. antarctica*. The digestive gland of both species is usually yellowish-orange or orange, but is dark brown immediately after feeding. Coloration in general is probably derived at least partly from diet, as starved animals lose the striking red color from the cones. The wings and integument are transparent, but slightly less so in specimens of *C. antarctica* which can exhibit some degree of opacity. Eliot (1907) stated that the integument of preserved specimens of *C. antarctica* contained more "yellow spots" than that of *C. limacina*. This statement apparently refers to oil droplets embedded in the integument, and Eliot's statement is true when comparing adults. However, polytrochous larvae and young juveniles of *C. limacina* are very similar to *C. antarctica* adults in having numerous oil droplets scattered throughout the integument.

Finally, the footlobes of *Clione limacina* tend to be short and wide. In comparison, all three lobes of *C. antarctica* are narrower and more elongate.

COMPARISON OF THE BUCCAL MASS

The feeding structures of *Clione* consist of three pairs of prehensile buccal cones (cephaloconi), paired hook sacs

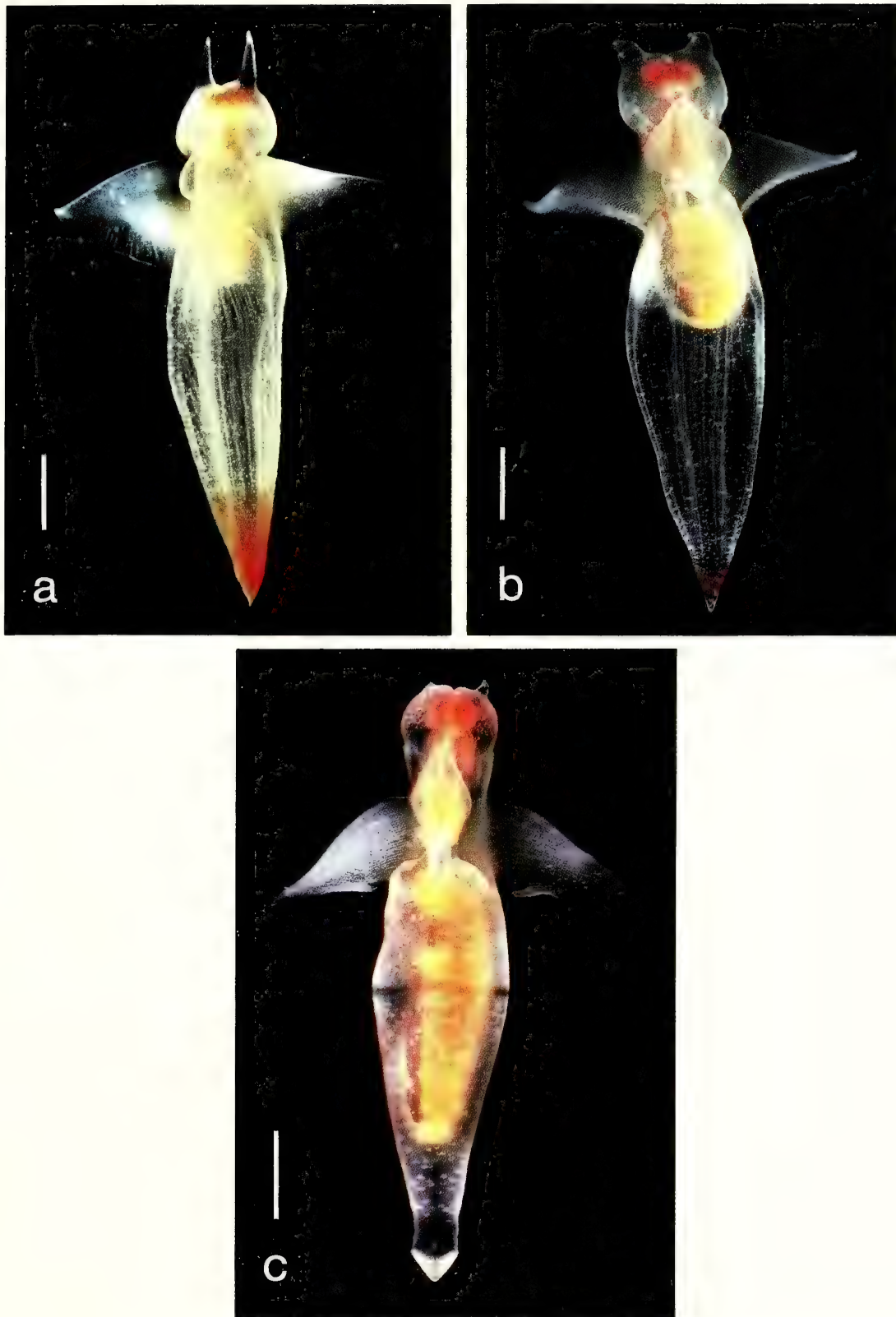


Fig. 1. Comparisons of adult *Clione* from different localities; all in ventral view: **a**, *Clione limacina* from the Subarctic Atlantic Ocean; **b**, *C. limacina* from the North Pacific Ocean; **c**, *C. antarctica* from McMurdo Sound (scale bars = 5 mm).

Table 1. A comparison of predator-prey maximal sizes in different locations.

Location	<i>Clione</i> Maximal adult length (mm)	<i>Limacina</i> Maximal adult diameter (mm)	Reference*
Subarctic Atlantic Ocean	70-85 (live)	11-12 (<i>helicina</i>)	2, 4, 8
Nova Scotia	20-25 (live)	<3.0 (<i>helicina</i> and <i>retroversa</i>)	2
English Channel	12	ca. 1.0 (<i>retroversa</i>)	5, 6
North Pacific Ocean	< 30 (live)	2.5 (<i>helicina</i>)	1, 7, 8
Central Arctic Ocean	70-80 (live)	ca. 11 (<i>helicina</i>)	3
Antarctic Ocean	42 (live)	5-6 (<i>helicina</i>)	8

*References

- | | |
|-----------------------------|---------------------------|
| 1 - Agersborg, 1923 | 5 - Lebour, 1931 |
| 2 - Conover and Lalli, 1972 | 6 - Lebour, 1932 |
| 3 - Ospovat, pers. obs. | 7 - McGowan, 1963 |
| 4 - Lalli and Wells, 1978 | 8 - Personal observations |

containing curved chitinous hooks, and the radula. There is no obvious morphological difference between the buccal cones of *Clione limacina* and *C. antarctica*. Meisenheimer (1906) and Eliot (1907) claimed that, in *C. antarctica*, the dorsal pair of cones was spatially separated from the other two pairs and that the medial cones were the largest; in contrast, the cones were said to be equidistant in *C. limacina*, with the dorsal cones being largest. We were not able to confirm this to our satisfaction. Instead, it would appear that size and relative placement of the buccal cones is dependent on their state of eversion or contraction in living animals, or on their degree of contraction in preserved specimens.

On the other hand, differences can be found in the structure of the chitinous hooks of the two species (Fig. 2). In all *Clione*, the hook sacs contain a variable number of hooks that can be everted from the sacs. These range in size from very small to fully developed, long, curved hooks. In the largest-sized adult *Clione limacina* from Subarctic waters of the North Atlantic Ocean, each hook sac contains approximately 60 hooks; these are arranged roughly in three rows, with the largest hooks being about 1.7 mm in length (Fig. 2a). In *Clione* from the North Pacific Ocean, the numbers and maximal size of the hooks are reduced, which is related to the smaller size of the species in this area; in adults, each sac contains about 30 hooks, with the largest about 0.7 mm in length. The hooks of *C. antarctica* are more numerous and larger relative to body size than those of *C. limacina*. In adult *C. antarctica*, each sac contains approximately 60 hooks, the largest is about 1.2 mm in length (Fig. 2b).

The most striking differences between the two species occur in the morphology of the radula. The radula of *Clione limacina* (Figs. 3, 4) consists of a relatively wide ribbon that extends dorsally and ventrally over the tip of the odontophore. Each row of chitinous radular teeth consists of a single, central, sickle-shaped tooth and, in our specimens, up to 12 curved, pointed, lateral teeth on each side. The number of rows of teeth and the number of teeth in each row varies with the size of the individual examined, and these numbers are maximal in the large Subarctic Atlantic Ocean specimens (Fig. 3a). However, apart from relative numbers of teeth and relative

size differences in the teeth, there is little difference in the structure of the radula and radular teeth between North Atlantic Ocean and North Pacific Ocean *Clione* populations. In all specimens of *C. limacina* that we examined, the central tooth is well developed, even in young specimens. Denticulation of the central tooth varies from sharp points on newly formed teeth (Figs. 3c, 4b) to low, broad protuberances on older teeth (Fig. 3b), indicating wear from use. There is also no discernible difference in the general shape of the lateral teeth between populations of *C. limacina* (Figs. 3a, 4a).

In contrast, the radula of *Clione antarctica* (Fig. 5) is extremely reduced, and this reduction is not proportionate to size differences between this species and the northern *Clione*. In Antarctic *Clione*, the minute radular ribbon is positioned differently on the odontophore. The ribbon splits just anteriorly to the radular sac and then extends forward as a broadening Y-shaped structure that spreads laterally over the tip of the odontophore (Fig. 5a). The lateral radular teeth (Fig. 5b) are smaller but similar in shape to those of *C. limacina*; the number of lateral teeth in each row is somewhat variable but is less than eight in all specimens examined. The most unusual feature is the absence of a central tooth. Although Eliot (1907) illustrated median teeth which he claimed were present in the first two rows of the radula, we have not been able to confirm this and concur with Pruvot-Fol (1932) who also did not find central teeth. Because of the lateral positioning of the ribbon on the odontophore tip, the only place where such teeth could be present would be immediately anterior to the radular sac (Eliot's "hindmost" rows). Examination with scanning electron microscopy (Figs. 5c, d), revealed that the bases of adjoining lateral teeth were attached and that these eventually pull away and separate as the ribbon splits laterally. We believe that the small point of attachment of the lateral teeth in this area was seen at lower magnifications by Eliot and confused with a denticle of a central tooth.

BEHAVIORAL COMPARISONS

All *Clione* feed on the thecosomatous pteropod *Limacina* by extracting the prey from its shell. In northern cold waters, *Clione limacina* feeds on *L. helicina* (Phipps), as does

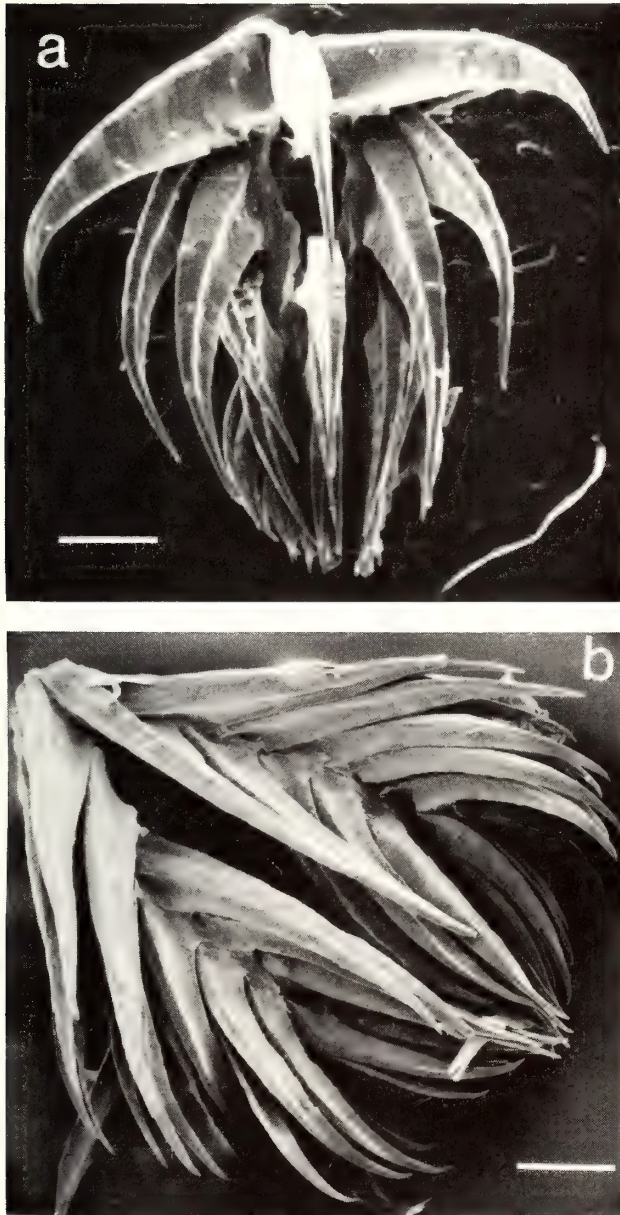


Fig. 2. Scanning electron micrographs of the hooks from a single hook sac: **a**, *Clione limacina* (North Atlantic); **b**, *C. antarctica* (scale bars = 50 μ m in **a**; 200 μ m in **b**).

C. antarctica in the Antarctic Ocean. In temperate waters of both the northern and southern hemispheres, *Clione* spp. co-exist with and feed on *L. retroversa* (Fleming). There are no significant differences in prey capture and feeding behavior between the two species of *Clione*. Maximal sizes attained by the predator and prey in different areas are linked (Table 1); both the prey and predator are largest in Subarctic waters and smallest in the English Channel. Antarctic Ocean species are intermediate in size. These size relationships in nature agree with the experimental laboratory results of Conover and Lalli (1972, 1974) suggesting that prey size over prey concentration and temperature is the major determinant of *Clione* size.

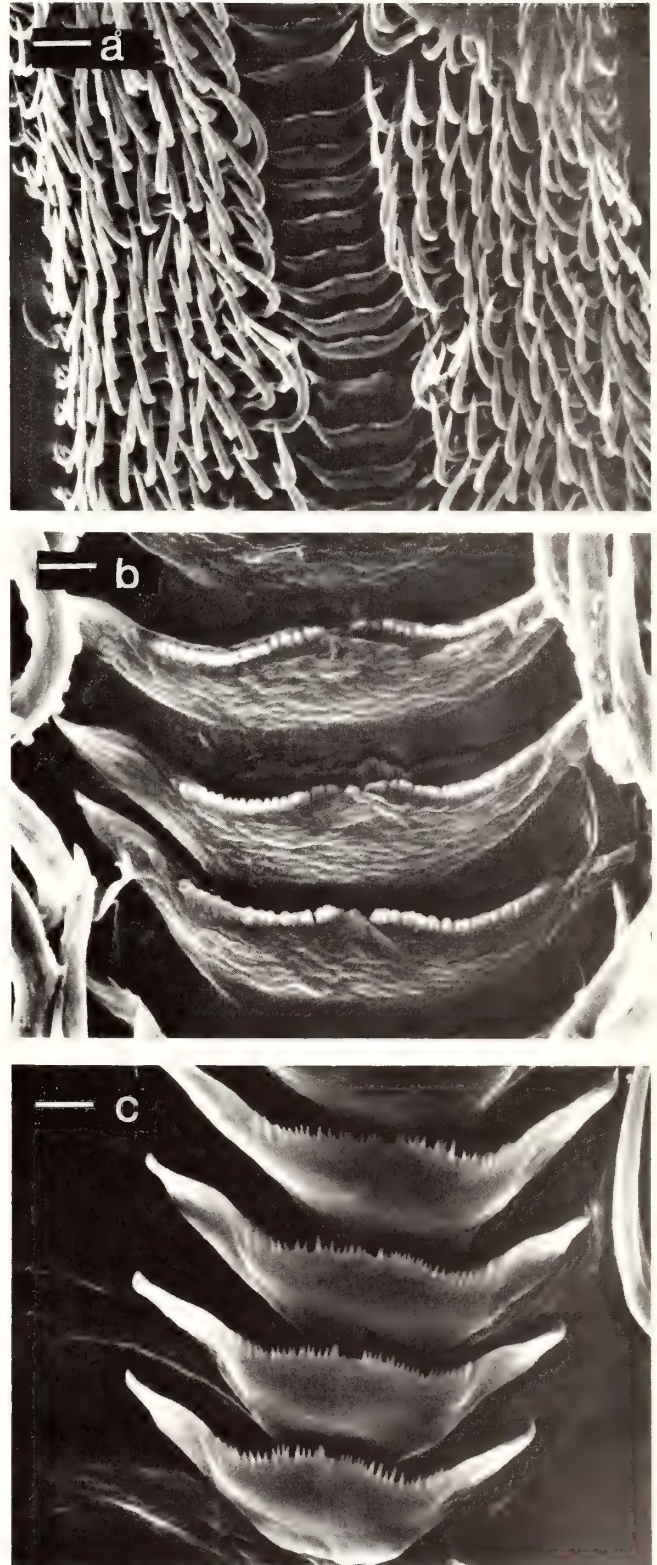


Fig. 3. Scanning electron micrographs of the radula of *Clione limacina* from the Subarctic Atlantic: **a**, median and lateral radular teeth; **b**, older median teeth showing wear; **c**, newly formed teeth (scale bars = 25 μ m in **a**, 5 μ m in **b** and **c**).

Swimming behavior of *Clione* has been observed *in situ* and in the laboratory. The majority of *Clione limacina* observed in the field are oriented head up and are simply maintaining their depth position with slow wing movements. During such periods of inactivity, subarctic specimens in water temperatures of less than 2°C move their wings dorsally and ventrally at a rate of about 2 beats/sec. The posterior tip of an animal is often curved ventrally at this time. When disturbed, the animals swim rapidly and the orientation of the body can be in any direction away from the disturbance. Individuals with body lengths exceeding 70 mm can swim at speeds of at least 10 cm/sec in waters of -0.5°C. The body of an animal often flexes dorsally and ventrally during rapid swimming and turning. Rapid wing motion can also be apparent during prey capture, but this does not necessarily result in movement. Slow, rhythmic swimming is continuous during mating, but rapid swimming in a circular pattern appears to be necessary to break the pairing. Spawning individuals swim slowly, moving their wings at about 2 beats/sec.

Clione limacina also appears to be able to achieve neutral buoyancy. On numerous occasions, divers have observed *C. limacina* hanging motionless in a head down position, with the wings extended. No measurable sinking occurs at such times.

In McMurdo Sound, Antarctica, *Clione antarctica* is generally found congregated near the undersurface of the ice shelf. It is generally sparsely distributed in water deeper than 20 m. Like *C. limacina*, this species also orients head up during slow rhythmic swimming, but it does not bend the posterior tip of the body ventrally. Wing motion is generally slow, at less than 2 beats/sec. When disturbed, however, large specimens (> 25 mm long) accelerate the wing beat to 4 to 5 beats/sec and can swim at speeds of about 5 cm/sec. This species reacts differently to disturbance. Most often, disturbed *C. antarctica* will stop swimming motions, retract the head slightly, and remain motionless in the water. Animals seldom attempted to swim away from a disturbance, which is the most common reaction of *C. limacina*.

Reproductive anatomy, copulation, spawning, and development are well known for *Clione limacina*, and these activities have been reviewed by Lalli and Gilmer (1989). Reproductive anatomy and behavior are not well known in the Antarctic species. Histological studies were made of four specimens of *C. antarctica* collected between November and January, when both spawning adults and veliger larvae were present. Specimens as small as 15 mm in length (preserved measurement) had oocytes in the gonad, sperm packed into a swollen genital duct, well developed mucous and albumen glands, and a large penis and prostrate gland. Two specimens of 30 to 40 mm length (live measurements) had spawned in the laboratory. Neither of these individuals showed any regression of copulatory structures and both had masses of sperm in the genital duct, indicating that they were capable of functioning as both male and female. However, copulation has never been observed in this species, either in the field or in laboratory-maintained individuals.

The size of the egg mass and the number of eggs spawned by three specimens of *Clione antarctica* varied with body size. The smallest individual (24 mm long, live measurement) produced an egg mass of 12 mm diameter containing 250 eggs. Two animals (33 mm and 38 mm long) spawned egg masses of 15 mm, with about 600 eggs produced by the smaller individual and about twice that many deposited by the larger animal. The largest egg mass was kept in the laboratory at 2°C (approximately 2.5°C above ambient temperature), and developmental times were recorded. On 12 November 1987, 24 hr after spawning, 20% of the eggs were at the second cleavage. At 400 hr, most embryos were spinning inside their egg capsules. About 10% had hatched as free-swimming veligers by approximately 20 days after spawning.

Veligers of *Clione antarctica* (Fig. 6) are characterized by a bilobed velum and a small shell with two distinctive areas. The cap-like embryonic portion of the shell measures about 110 μ m long and 160 μ m at its widest diameter. The post-embryonic extension of the shell, which is formed after

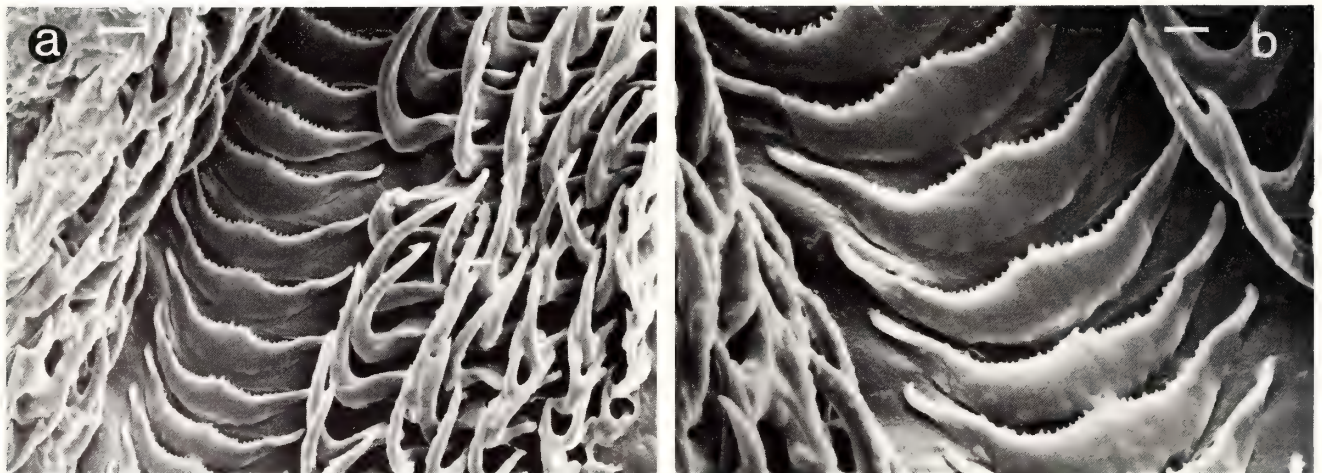


Fig. 4. Scanning electron micrographs of the radula of *Clione limacina* from the North Pacific: a, median and lateral radular teeth; b, median teeth (scale bars = 10 μ m in a, 5 μ m in b).

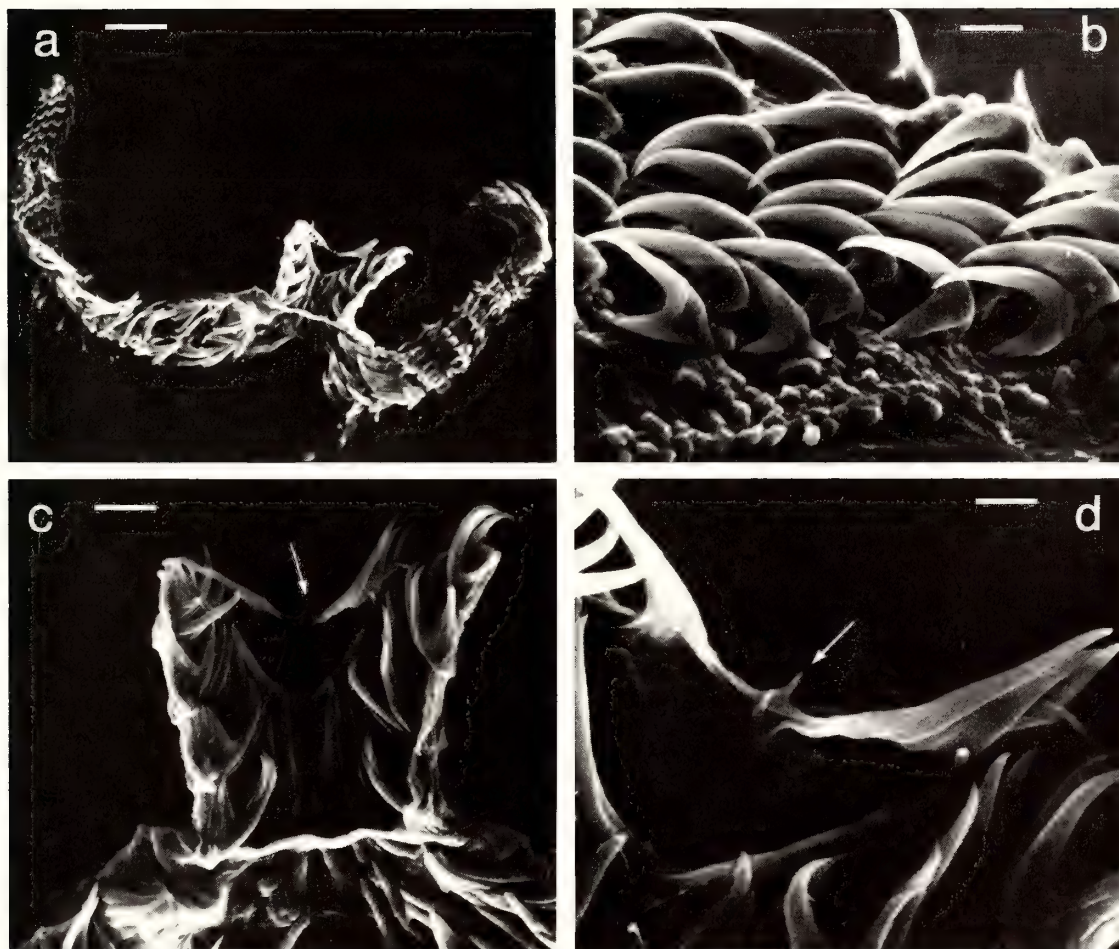


Fig. 5. Scanning electron micrographs of the radula of *Clione antarctica* from McMurdo Sound: a, configuration of the complete radula; b, lateral radular teeth; c, adjoined lateral teeth in the median furrow (arrow indicates fused teeth); d, enlargement of c (scale bars = 40 μ m in a, 8 μ m in b, 12 μ m in c, 4 μ m in d).

hatching, flares out like a collar from the embryonic shell and has distinctive, encircling growth rings. The shells differ from those of *C. limacina* veligers (see Lalli and Conover, 1976) in being more rounded posteriorly, with a shorter and broader embryonic portion. We have not been able to determine the size at which the larval shell is cast, nor have we observed loss of the velum and metamorphosis to the polytroch stage.

The polytrochous larvae of *Clione antarctica* are similar to those of other gymnosome species in having three ciliary bands which encircle the body, but they already show the distinctive traits that separate them from the northern *Clione*. A comparison of the polytroch larvae (each 9 mm long) of *C. antarctica* and of *C. limacina* from the North Pacific (Fig. 7) shows the striking differences in head to body proportions and in footlobe shape that are also present in the adults. It is also clear that the protuberances underlying the cilia of the anterior larval band are much more prominent in *C. antarctica* larvae. At this stage, the Antarctic polytrochs already closely resemble the adults; the only major anatomical change that will accompany growth will be the gradual regression



Fig. 6. Veliger larva of *Clione antarctica* (scale bar = 50 μ m).

of the cilia in the larval rings. The polytrochous larvae of *C. limacina*, however, will undergo a more dramatic metamorphosis with rapid and complete loss of the anterior and middle ciliary bands, more gradual loss of the posterior ciliary ring, and a progressive lengthening of the trunk until the viscera are confined to the anterior half of the body.

DISCUSSION

Meisenheimer (1906) and Eliot (1907) were the first to present detailed accounts of *Clione* collected from the Antarctic. Both authors noted anatomical differences, but Meisenheimer preferred to regard these gymnosomes as a variety of *C. limacina*, whereas Eliot considered that the differences were such as to validate Smith's establishment of *C. antarctica*. Eliot presented a list of nine major anatomical distinctions, and several minor ones, between *C. antarctica* and *C. limacina*. We concur with many of his points concerning *C. antarctica*: the head is larger relative to body length; the body of the Antarctic species is smaller; at least two larval bands are retained into the adult stage; the viscera extend farther posteriorly; and the footlobes have a different shape and a narrower attachment. We are hesitant to accept that the buccal cones have a different arrangement, as this seems to be a preservation artifact. Nor do we agree that there are more oil droplets in the integument than there are present in *C. limacina*; this appears dependent on the size

(and age) of the animals which are compared. However, there is a tendency of Antarctic Ocean animals to develop a slight opacity of the integument compared to northern ones. On the other hand, we concur with Pruvot-Fol (1932) that Antarctic Ocean specimens do have a well-developed copulatory organ, consisting of a penis, prostrate gland and accessory organ, that is identical to that of *C. limacina*. We also share her view that median radular teeth are absent in *C. antarctica*, in contrast to the well-developed and conspicuous median teeth found even in very small specimens of *C. limacina*. In addition, our results show that the hooks of Antarctic Ocean animals are larger and more numerous relative to body size; this is probably correlated with the diminution of the radula and probably indicates a greater involvement of the hooks in extraction of prey from its shell.

Behavioral differences between the two species do not appear to be so pronounced, but this could be due to fewer hours of observation of living *Clione antarctica*. Both *C. limacina* and *C. antarctica* feed on *Limacina helicina* or *L. retroversa*; we have not observed any significant difference in prey capture or in ingestion time from that described by Lalli (1970) and Conover and Lalli (1972). The Antarctic Ocean species, however, swims at slower rates than does its northern counterpart, and it usually responds to disturbance by cessation of swimming rather than active movement away from a stimulus. Both clionids deposit free-floating, gelatinous egg masses. The size of egg masses and the number of eggs per mass are smaller in *C. antarctica* than in large, subarctic *C. limacina*, but are larger than in dwarf specimens of *C. limacina* from the English Channel (Lalli and Gilmer, 1989). In the northern species, hatching of the veligers coincides with periods of maximal phytoplankton abundance and with simultaneous hatching of *Limacina* veligers (Mileikovsky, 1970; Conover and Lalli, 1972). In the Antarctic Ocean, we have collected spawning adults from November through January, and Massy (1932) reported finding larvae of less than 3 mm length in October, November, December and February. There are differences in the size and shape of the veliger shell of *C. antarctica*, as well as between the polytroch larvae of the northern and southern species. It is interesting that *C. antarctica*, like several other gymnosome species (Lalli and Gilmer, 1989), displays neotenous characters, retaining external larval features and a relatively small size after reaching sexual maturity. In contrast, *C. limacina* usually undergoes a complete metamorphosis from the polytroch stage; a posterior larval band is present only in the small-sized individuals living in the English Channel (Lebour, 1931; Morton, 1958) or in the rare neotenous individual collected off Nova Scotia (Lalli and Conover, 1973).

Although we do not agree on all the points of difference between northern and southern *Clione* as established by earlier workers, we do believe that the evidence presented here further strengthens the taxonomic distinctions separating *C. limacina* and *C. antarctica*. Although both clionids occupy identical ecological niches in cold water areas, feeding on two species of the shelled pteropod *Limacina* and exhibiting similar behavioral patterns, they are sufficiently different morphologically to justify their separation. In addition, the

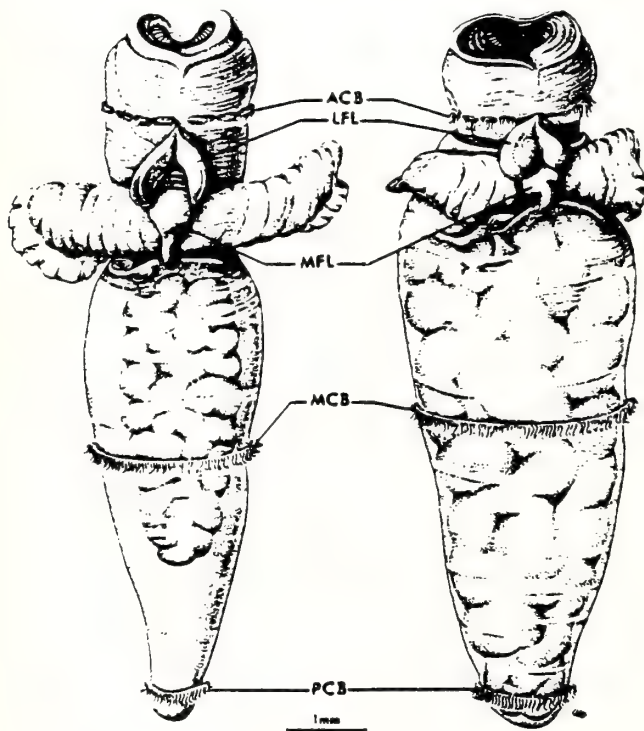


Fig. 7. Polytrochous larvae of *Clione antarctica* from the Antarctic Ocean (left) and of *C. limacina* from the North Pacific (right) (ACB, anterior ciliary band; LFL, lateral footlobe; MCB, median ciliary band; MFL, median footlobe; PCB, posterior ciliary band).

species are spatially isolated. There is no evidence to suggest that there is any physical connection between the northern and southern populations, so there is no possibility of interbreeding. However, it is evident from the similarity of the species in the two hemispheres that they have evolved from a common ancestor.

ACKNOWLEDGMENTS

We wish to acknowledge and thank Dr. T. R. Parsons for laboratory supplies and facilities and Dr. G. R. Harbison for shiptime on two Subarctic cruises in the North Atlantic. We also gratefully acknowledge the technical assistance of M. Weis, P. Linley, T. Smoyer, and P. Blades-Eckelbarger. Thanks are also due to G. Harbison, J. Lindsey, G. Dietzmann, D. Backus, and N. Wu for diving and photographic assistance, and to M. Ospovat for his diving observations in the Central Arctic Ocean. Research support for RWG was provided by N.S.F. grants OCE-8209341, 8516083, 8746136 and DPP8613388 to G. R. Harbison; we also acknowledge research support from NSERC grant 0006689 to T. R. Parsons. This is Harbor Branch Oceanographic Institution Contribution No. 749.

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Date of manuscript acceptance: 3 October 1989.

ANNUAL CYCLE OF *LIMACINA RETROVERSA* IN PATAGONIAN WATERS

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ABSTRACT

An annual cycle (April 1978 - April 1979) of *Limacina retroversa* (Fleming) in Patagonian shelf and surrounding waters (40° - 55°S) is analyzed. Both abundance and mesoscale distribution of this species in the area were strongly associated with the seasonal cycle. A stationary distribution pattern was evident in autumn and winter. The shelf population concentrated chiefly in a dense aggregation (maximum: 67,800 individuals per 1,000 m³) located to the northwest of the Malvinas Current. Individuals expatriated northward connected this aggregation with others carried by the core of the Malvinas Current. In October, the aggregation on the shelf began to disintegrate and proceeded slowly northward and off the shelf. The summer distribution pattern consisted of scattered individuals on the shelf and some aggregations moving along the slope.

Hydrological dynamics together with diel vertical migrations appeared to be of crucial importance in maintaining the observed distributions over shelf waters.

As with many other groups of marine zooplankters, pelagic mollusks have been little studied in the Southwestern Atlantic. Boltovskoy (1973, 1975) and Magaldi (1974, 1977) completed an inventory of the existing species and related the faunistic assemblages with megascale hydrological characteristics. However, their conclusions are based on a relatively small number of samples, which were not quantitatively, discontinuous in time, and obtained mainly from a reduced area between 35° and 40°S.

In Patagonian waters south of 40°S, only subantarctic species can be found (Dadon, 1984, 1986). Though poor in diversity, pelagic mollusks in these cold waters are abundant, reaching densities of 55,000 per 1,000 m³ on the slope and outer shelf during winter. *Limacina retroversa* (Fleming) was found to be the predominant species; the other planktonic mollusks were *L. helicina* (Phipps) and *Clio antarctica* Dall.

Seasonality in hydrographic conditions and biological production is very well known in Patagonian waters. Primary production peaks in September, October or November, depending on the latitude (Mandelli and Orlando, 1966; Carreto *et al.*, 1981a). Zooplankton biomass increases in spring, following the period of high phytoplankton production (Ciechomski and Sanchez, 1983). This study is mainly concerned with seasonal patterns of *Limacina retroversa* in Patagonian waters. Monthly mesoscale distribution and changes in total abundance of the species during an annual cycle are analyzed and hypotheses are developed concerning the role of

biological and environmental factors.

MATERIALS AND METHODS

Eighteen exploratory cruises were conducted in shelf and slope waters off Patagonia between April 1978 and April 1979 by the R/V "Walther Herwig" and "Shinkai Maru" (see Ciechomski *et al.*, 1979; Cousseau *et al.*, 1979). These collections included 539 samples taken to the south of 40°S, all of which were analyzed for *Limacina retroversa*.

Samples were collected during daytime and twilight with Bongo nets (mouth diameter = 60 cm; length = 330 cm) fitted with 330 or 505 µm mesh nets. On the continental shelf, oblique tows extended from the surface to approximately 20 m above the bottom; on the slope, from the surface to 100 m depth. The maximal tow depth was estimated by time-depth recorders. The nets were provided with digital flow meters in both mouth openings and were towed at 3.5 knots. In all cases, the volume of water filtered through the nets was 200 - 1000 m³.

For counting, the samples were divided in aliquots with a Folsom plankton sample splitter until the final aliquot contained 180 to 300 individuals. Sparse samples were analyzed in their entirety.

For large volumes, little or no significant differences were obtained either in the plankton volume or in the size composition of the zooplankters, between the plankton volume filtered by the two meshes (Ciechomski and Sanchez, 1983).

HYDROLOGY

The Patagonian continental shelf is broad, with a gradual slope. Neritic waters are mostly of subantarctic origin with some admixture from continental run-off. Surface temperatures varied from 2.3 to 20.2°C; in general, temperature increased northward and westward. Surface salinity varied from 34.17 o/oo (slope waters) to 32.39 o/oo (inner shelf waters). For detailed information about the physical data of the studied cruises see Ciechomski *et al.* (1979) and Cousseau *et al.* (1979).

The mesoscale hydrology of the area is dominated by the Malvinas or Falkland Current, which is a branch of the West Wind Drift (Fig. 1). Satellite observations (Legeckis and Gordon, 1982) show the Malvinas Current to be a well defined, 100 km-wide belt of cold waters. The core of this current runs close to the western boundary of the slope. Surface waters overlying the shelf also flow in the same general direction as the Malvinas Current, but at a considerably lower velocity. From the Gulf of San José (approximately 45° and 47°S) to the slope, the Patagonian Coastal Current (Brandhorst and Castello, 1971) flows Northeast through the shelf (Fig. 1).

The contact between the Malvinas and Brazil currents constitutes the Subtropical-Subantarctic Front. This Front is located to the north of the Patagonian waters (Fig. 1) and constitutes a complex zone. It marks the limit of the distribution

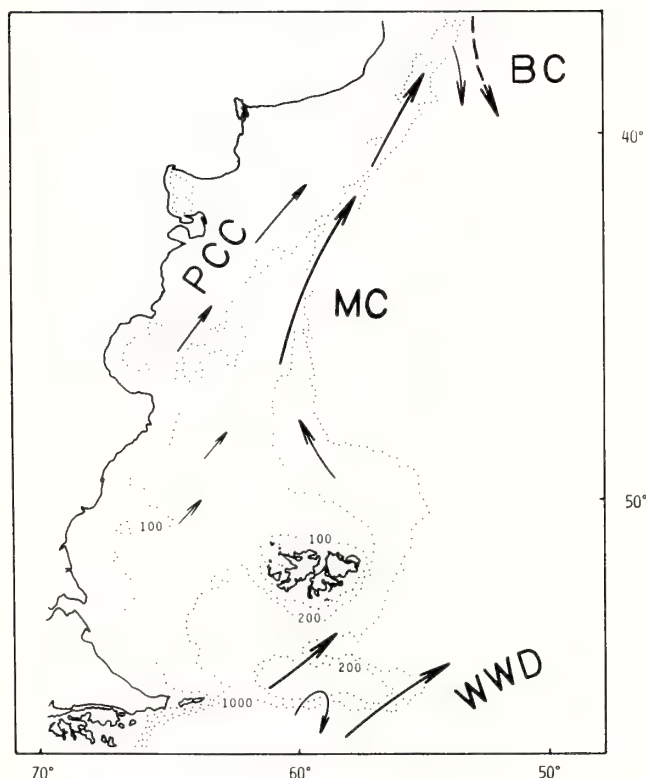


Fig. 1. General hydrology of the study area (cold waters, full line; WWD, West Wind Drift; MC, Malvinas Current; PCC, Patagonian Coastal Current; Warm waters, hatched line; BC, Brazil Current).

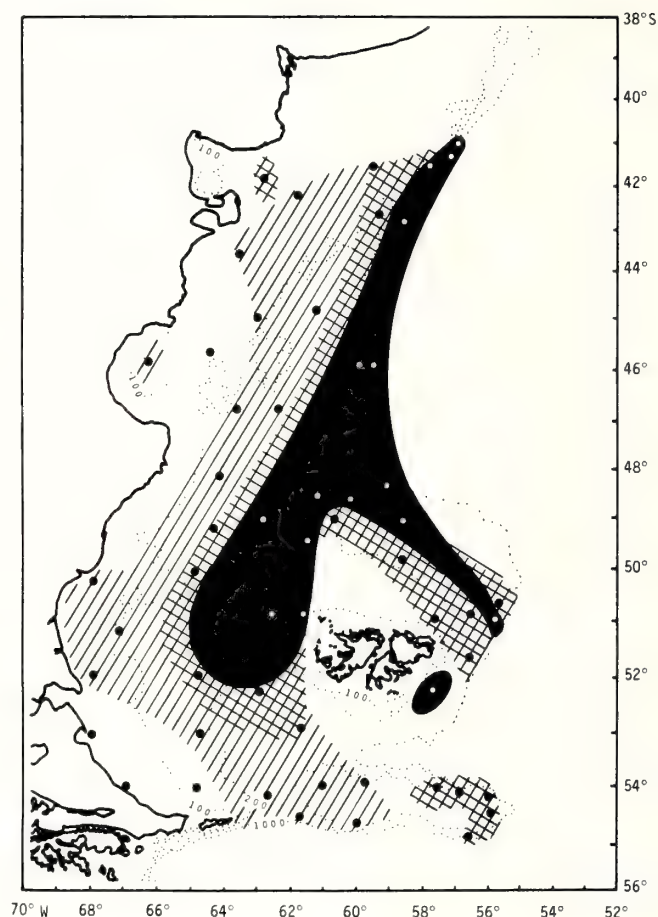


Fig. 2. Distribution of *Limacina retroversa* in May 1978. (Circles denote stations. Abundances as follows: Black areas indicate >1,000 per 1,000 m³; cross-hatched areas, 1,000 - 100 per 1,000 m³; hatched areas, 100 - 10 per 1,000 m³; white areas (when sampled) <10 per 1,000 m³).

of the subantarctic fauna in shelf waters and its position varies seasonally (Tseng, 1974; Olson *et al.*, 1988).

RESULTS

SEASONAL DISTRIBUTION

The mesoscale horizontal distribution of *Limacina retroversa* in Patagonian waters showed a well defined pattern during the 1978 - 1979 annual cycle (Figs. 2-6). Densities higher than 1,000 individuals per 1,000 m³ (maximum of 67,800 per 1,000 m³) were found in slope waters throughout the year and in intermediate and outer shelf waters from April to September, 1978. In slope waters, dense aggregates were recorded that were transported northward by the Malvinas Current throughout the year (Figs. 2, 3, 6). In general, aggregates traveling in slope waters seemed to replace each other easily and quickly. Other authors (Bigelow, 1926; Redfield, 1939) pointed out the tendency of *L. retroversa* to aggregate.

The western limit for massive entrance of *Limacina*

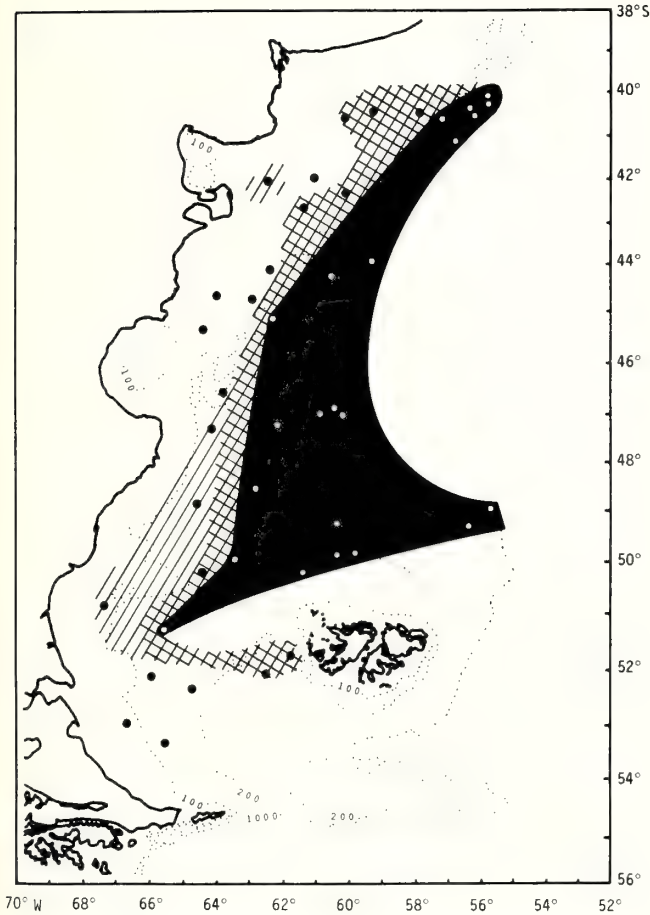


Fig. 3. Distribution of *Limacina retroversa* in June 1978. Symbols as in figure 2.

retroversa to the shelf coincided with the 100 m isobath to the south of 41°S (Figs. 3, 5). Between this dense strip and the coast, low densities were only occasionally registered, especially to the north of 47°S (Figs. 3, 4, 5). This low density or absence of the species constituted a tongue-like area which coincided with the path of the Patagonian Coastal Current. The holoplanktonic fauna depicted for this current is predominantly inner neritic [i.e. *Sagitta friderici* Ritter-Zahony, 1911 (Dadon and Mazzoni, 1989)]. Taking into account the geographical range of *L. retroversa* in the area, this species is oceanic but can tolerate neritic conditions.

In shelf waters, the highest densities were distributed in an oblong or pear-shaped configuration during the austral autumn and winter (Figs. 2, 3). The widest portion was located to the west of the Malvinas Islands and was continued as a band in a NNE direction onto the slope. This distribution was maintained without important changes practically from May until the beginning of spring. The only variations during this period were deformations and slight displacements of the aggregation towards the west (cf. Fig. 2 vs. Fig. 3). Same variations were observed for *Limacina helicina*, chaetognaths and cladocerans patterns (Dadon, 1986). Ramirez (1981) and Carreto *et al.* (1981b) pointed out that in winter, oceanic

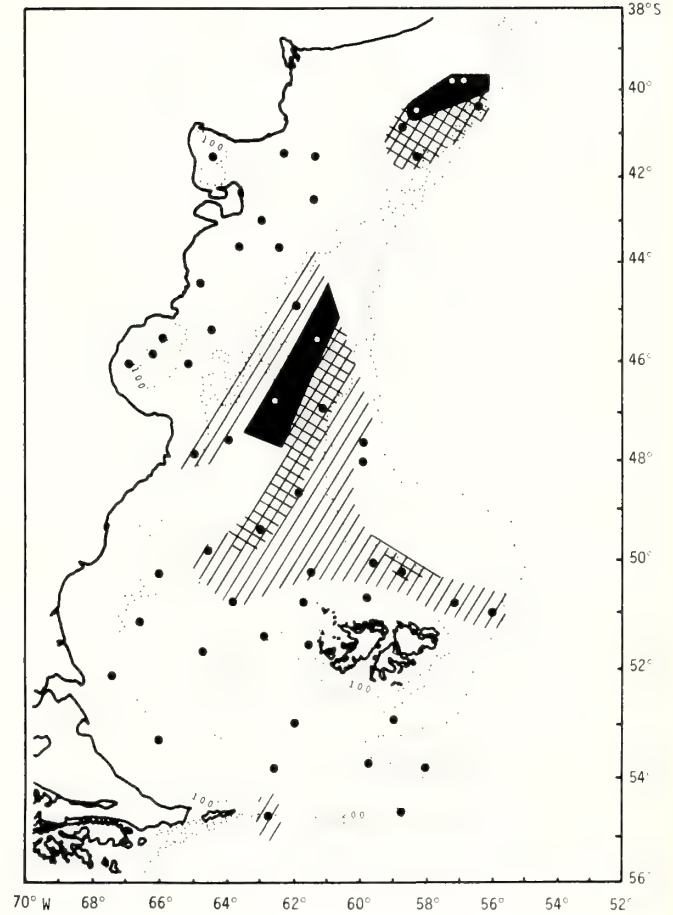


Fig. 4. Distribution of *Limacina retroversa* in October 1978. Symbols as in figure 2.

euphausiids, amphipods and copepods occurred predominantly on the Patagonian shelf, while typical neritic zooplankters were found in more offshore waters.

At the beginning of spring, a series of important changes in the distribution of *Limacina retroversa* on the continental shelf were observed (Fig. 4). The oblong aggregation began to disintegrate. Each fragment was carried to the northeast, reaching the slope at latitudes below 40°S (Dadon, unpub. data). At least two of those isolated fragments were detected in October (Fig. 4), and two or three of them in November (Fig. 5). This process continued up to March 1979, when the last important concentration of organisms on the shelf was recorded between 43 and 45°S (Fig. 6). The summer distribution pattern consisted of scattered individuals on the shelf and dense aggregations moving along the slope without penetrating the shelf, as shown between 39° and 42°S in figure 6.

DENSITY IN RELATION TO ENVIRONMENTAL FACTORS

Limacina retroversa was found in waters when the surface temperatures ranged from 4.1 to 18.8°C, and surface salinities were between 32.39 and 34.17 ‰. The temperature

interval where the species was recorded in the Patagonian shelf waters (4 - 19°C) was almost the same as the one recorded for the Southwestern Atlantic (see Spoel and Boltovskoy, 1981). Although densities higher than 1,000 per 1,000 m³ were found at 4.5 - 17.8°C and 33.07 - 34.15 o/oo, this range was mainly recorded at 5 - 9°C and > 33.50 o/oo. These optima differ from those reported for northern hemisphere populations of *L. retroversa*: 8 - 10°C and 34.5 - 35.0 o/oo in the North Atlantic (Chen and Bé, 1964); 7 - 12°C in the Gulf of Maine (Bigelow, 1926); 7.5 - 10°C in the Northeast Atlantic (Beckmann *et al.*, 1987).

To obtain a simple relation between selected environmental factors and the species density in a given area, empirical relationships were sought. This analysis does not assume a direct cause-effect relationship, but, as Haedrich and Judkins (1979) pointed out, it constitutes a first necessary approximation to a very complex problem. Spatial variation in the density of *Limacina retroversa* density was compared to several environmental variables (depth, temperature, salinity and oxygen concentration) recorded during Cruises I and II of R/V "Shinkai Maru" (Cousseau *et al.*, 1979). Association between density and each variable was determined by correlation analysis (Sokal and Rohlf, 1981). Data were nor-

mally distributed in all cases except for depth and density, for which the transformations $x' = x^{1/2}$ and $x' = \ln(x + 1)$ were applied, respectively.

The abundance of *Limacina retroversa* and the depth are positively correlated (Table 1), indicating that these organisms are denser in deeper waters. The correlation between density and temperature is negative, indicating that *L. retroversa* has a higher affinity for cold waters. However, the correlation between density and temperature decreases at greater depths. The strongest correlations between temperature and density were for the average temperature and for temperatures of the upper layers (surface to 20 - 25 m).

Confronted with the other environmental factors (Table 1), salinity shows lower correlation coefficients with density ($r < 0.39$; $P < 0.05$ or not significant). Dissolved oxygen concentration and density were positively correlated in all cases and, in contrast to temperature and salinity, the average did not show a higher value than its components when considered individually. The highest correlation values were achieved for oxygen concentration at depths between 0 and 45 - 50 m.

In order to establish the maximum proportion of the observed variation in the density of *Limacina retroversa* which can be explained in terms of the environmental variations, the

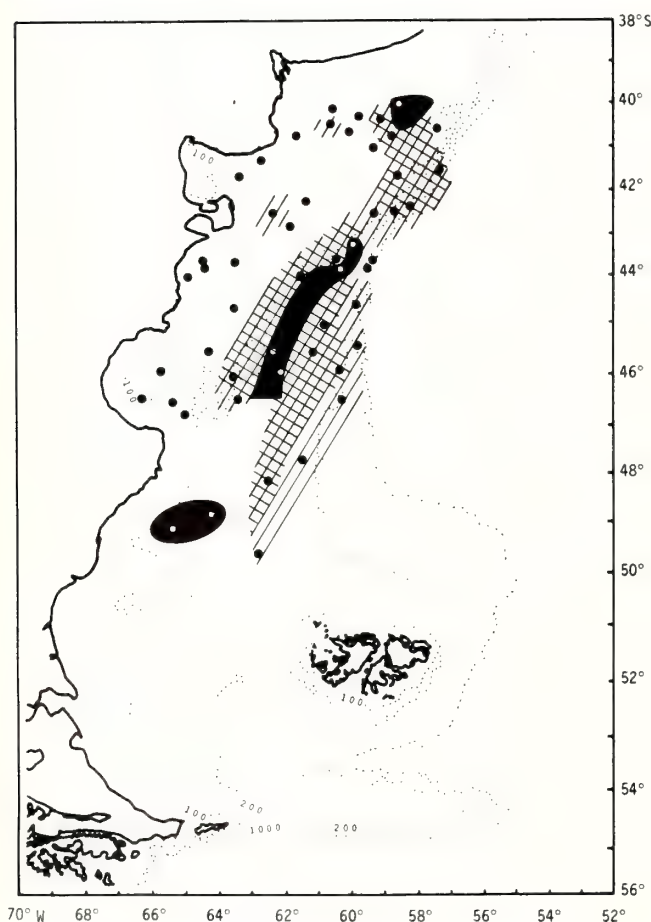


Fig. 5. Distribution of *Limacina retroversa* in November 1978. Symbols as in figure 2.

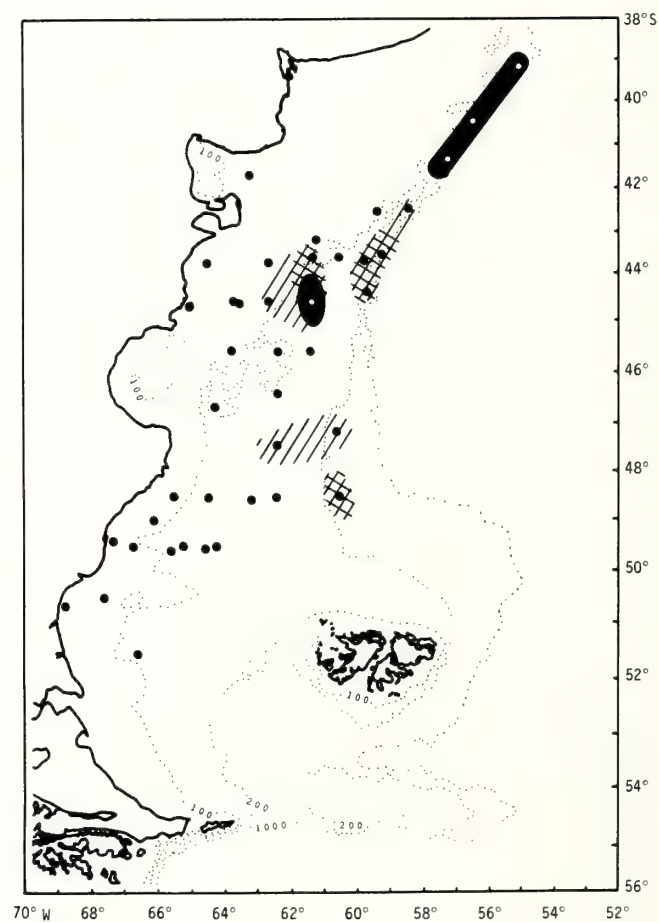


Fig. 6. Distribution of *Limacina retroversa* in March 1979. Symbols as in figure 2.

backward elimination and the forward selection procedures (see, for example, Drapper and Smith, 1966) were carried out considering the 25 environmental variables (Table 1) as predictors. The best regression equation was

$$Y = -90.748 + 4.308 X_1 + 2.036 X_2 \quad R^2 = 0.52$$

where Y: density of *L. retroversa*; X_1 : oxygen concentration at 20 m; and X_2 : average salinity. Since the most commonly-measured factors are temperature and salinity at the surface, their efficiency in predicting the species density either individually or in combination was analyzed for all months. Coefficients of determination ranked from 0.001 to 0.48 in all cases.

DISCUSSION

FACTORS GENERATING AND MAINTAINING MESOSCALE DISTRIBUTION

Detailed analysis of distribution and abundance patterns implies the study of the relations between the organisms and the environment. From Pickford (1946) on, several authors have repeatedly contended that the distribution of plankton is primarily ruled, or at least potentially governed, by the distribution of the water masses that the plankters inhabit. In certain cases, clear evidences in favor of this hypothesis were provided (see the review of Haedrich and Judkins, 1979; in pelagic mollusks, Furnestin, 1978). In other cases, this relation could be demonstrated only when intraspecific variations were considered (i.e. McGowan, 1963). In yet other cases, although the same species inhabited more than one water mass, there were remarkable differences in the density of individuals present in each environment, pointing out the necessity for quantitative studies when faunistic areas are to be compared (Fasham and Angel, 1975; Dadon, 1984). In many cases, however, this hypothesis had to be rejected because the species were ubiquitous or were highly cosmopolitan.

Limacina retroversa inhabits quite different water masses in the Southwestern Atlantic. It was collected on both sides of the Antarctic Convergence (Chen, 1968) and its geographical range extends northward to the Subtropical - Subantarctic Front. However, quantitative analyses (e.g. Bé and Gilmer, 1977: Fig. 7) have revealed that the area of highest densities is much less extensive and confined to the subantarctic region. Despite this clear association between *L. retroversa* and subantarctic waters, the present analysis of mesoscale distribution showed that the relationship is not simple and that it depends on several factors. As above mentioned, high densities of *L. retroversa* in patagonian waters were correlated with highly oxygenated, cold pelagic (= deep) waters. This set of correlations clearly defines the core of the Malvinas Current as the most favorable habitat in the area. This current is probably the only way for *L. retroversa* to enter the region, although the aggregates recorded in the core (i.e. in slope waters) seem to be rapidly expatriated northward. High densities associated with shelf waters, even when influenced by the Malvinas Current, established a stable distribution during autumn to winter. This stability implies the

Table 1. Correlation between density of *Limacina retroversa* and environmental variables (TD, tow depth; WA, weighted average for the sampled water column; r, correlation coefficient; **, $P < 0.01$; *, $P < 0.05$; NS, $P > 0.05$).

Environmental Variables	r	
Depth (m)	0.5376	**
Temp (°C) 0 m	-0.6252	**
" 10 m	-0.6276	**
" 20-25 m	-0.6188	**
" 45-50 m	-0.5316	**
" 65-70 m	-0.3351	*
" 90-100 m	-0.3122	*
" TD m	-0.6166	**
" WA	-0.6546	**
Salinity (‰) 0 m	0.3166	*
" 10 m	0.3206	*
" 20-25 m	-0.2140	NS
" 45-50 m	0.3408	*
" 65-70 m	0.2701	NS
" 90-100 m	0.3101	NS
" TD m	0.2448	NS
" WA	0.3898	*
O ₂ Conc (mg/l) 0m	0.6416	**
" 10 m	0.6320	**
" 20-25 m	0.6557	**
" 45-50 m	0.6372	**
" 65-70 m	0.5359	**
" 90-100 m	0.5605	**
" TD m	0.5276	**
" WA	0.5814	**

existence of steady environmental conditions, responsible for generating and maintaining (or at least, allowing) such a pattern.

Since plankton is transported passively, it is necessary to look for dynamic aspects of the oceanic environment. In an open area like the Patagonian shelf, a stable distribution necessarily implies a closed flow preventing massive, short-term expatriation of the organisms. *Limacina retroversa* inhabits the upper 150 m of the water column (Bé and Gilmer, 1977). Superficial hydrology of the area is predominantly unidirectional, i.e. through the area from south to north as an open flow. Conversely, deep layers are expected to flow at different velocities and directions.

While on the slope the Malvinas Current moves fast and unidirectional at all depths, on the shelf there are differences in the movements of the waters at different levels, as indicated by the theoretical winter current field calculated by Lusquinos and Schrott (1982). Between the surface and 20 m, current vectors on the continental shelf have a direction exclusively NNE, but at deeper levels (> 30 m), velocity decreases sharply and even reverses turning to the Southwest; this reversal is more important over the outer shelf south of 46°S (Fig. 7). Comparing the winter distribution pattern of *L. retroversa* with this current field, the accumulation zone can be seen to lay within the area where the direction of the flow reverses. Diel vertical migrations of *L. retroversa* have been observed by several investigators (Bigelow, 1926; Chen and Bé, 1964). Thus, individuals migrate through layers

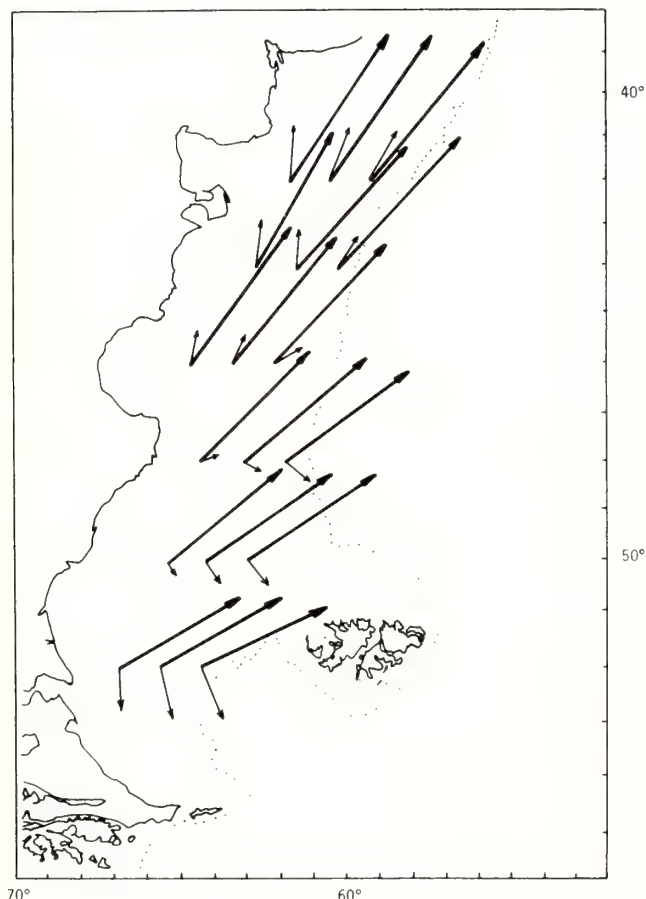


Fig. 7. Theoretical winter field of currents (according to Lusquinos and Schrott, 1982) (thick arrows, 0 m; thin arrows, 50 m).

which move at different velocities and even in opposite directions. The bulk of the individuals in the area prevent massive expatriation, retrogressing during the day the way they progressed during the night. On the other hand, irreversibly expatriated individuals would gradually approach the continental slope, as a band connecting the aggregation area and the slope (Figs. 2, 3). This mechanism would explain the shape and the long-term stability of the aggregate on the continental shelf.

SEASONAL CHANGES

Typically, a marked seasonality is shown in Patagonian waters, both for environmental and biological factors. During autumn and winter, the cooling of waters is uniform and smooth; on the contrary, warming of the water column during the warm period is sharp (Krepper and Bianchi, 1982). Temperature gradients between the coast and the slope became more distinct during spring and summer than the ones observed during fall and winter (Branshorst and Castello, 1971; Legeckis and Gordon, 1982). The primary production peaks during September in the northern area and this pulse displaces to the south reaching the southern extreme in November (Carreto *et al.*, 1981a). The highest chlorophyll con-

centrations (100 - 200 mg/m²) were detected between 44° and 47°S (Carreto *et al.*, 1981a). Zooplankton production follows a similar pattern, though slightly delayed (Ciechomski and Sanchez, 1983). During the 1978 - 1979 cycle, the highest zooplankton densities were registered by Ciechomski and Sanchez in November in the northern portion (42 - 44°S) and in summer in the south (51 - 53°S). Evidences of massive spawning in shelf waters during November 1978 were also found for oceanic zooplankters which, like *L. retroversa*, are transported to the outer shelf by the Malvinas Current (e.g. euphausiids; see Ramirez and Dato, 1983). According to this, a springtime pulse of *L. retroversa* should be expected coinciding with this increase in general zooplankton production.

After November, the abundance of *L. retroversa* diminished as well as the area occupied by the species on the continental shelf. The last remnants of the dense core which had occupied the major part of the continental shelf were detected towards the end of summer, in March 1979, between 43 and 45°S (Fig. 6). Over the rest of the shelf, and especially in the southern portion, *L. retroversa* was present only occasionally and always in very low densities (< 10 per 1,000 m³). Nonetheless, even when abundance over the shelf was lower in April 1979, densities up to 100 per 1,000 m³ were detected in neritic waters south of 47°S, indicating the beginning of a new cycle. According with these observations, not only the reproduction but also the massive immigration seem to show a seasonal pattern.

Seasonal patterns in abundance and/or horizontal distribution have been previously described for *Limacina retroversa* in other regions (Redfield, 1939; Vane and Colebrook, 1962; Paranjape and Conover, 1973). The annual cycle of this species on Patagonian shelf waters shows some similarities with that described by Redfield (1939) for the Gulf of Maine. In both areas, *L. retroversa* is alternately repatriated and expatriated in large numbers and cannot be maintained from one year to the next by means of *in situ* reproduction alone. The dynamics for this species and, probably, for most oceanic species of zooplankton, is predominantly regulated by migratory events. This is clearly evident in the core of the Malvinas Current (on the continental slope), where aggregations are rapidly transported northward, but also in shelf waters. In the latter, even when seasonal changes in the local hydrology allow a temporary residence, and eventually reproduction, they cannot preclude a final expatriation. In this context, the Patagonian waters can be described as a subsidiary system, a one-way transit via connecting the West Wind Drift with the Transition Zone in which the long-term presence of the oceanic zooplankton depends on the production surplus coming from other systems.

ACKNOWLEDGMENTS

The author wishes to express his thanks to the Instituto Nacional de Investigación y Desarrollo Pesquero (Mar del Plata, Argentina) and especially to Juana D. de Ciechomski, for lending the samples; to Demetrio Boltovskoy, for his constant support and his critical revision of the manuscript; to Beatriz Gonzalez, for the revision of the statistical methodology; and to Roger R. Seapy, for his valuable suggestions and styling of the English version.

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Date of manuscript acceptance: 2 May 1990.

THE TAXONOMY, DISTRIBUTION AND BIOLOGY OF *ATLANTA GAUDICHAUDI* SOULEYET, 1852 (GASTROPODA, HETEROPODA) FROM THE GREAT BARRIER REEF, AUSTRALIA

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ABSTRACT

Investigations into the holoplanktonic gastropod fauna of the Great Barrier Reef, from 1985-1988, showed that *Atlanta gaudichaudi* Souleyet, 1852 was the most common atlantid species found within the surface waters around Lizard and Heron islands. The taxonomy of this species is reviewed particularly with respect to two morphologically related species, *A. peroni* Lesueur, 1817 and *A. plana* Richter, 1972. Veligers of *A. gaudichaudi* are described for the first time. The mean abundance of *A. gaudichaudi* ranged from 0.2 to 17.3 animals/m³. Abundances were higher at Heron Island (subtropical waters), especially in summer months. At Lizard Island (tropical waters) greatest numbers were found during the winter. Over 70% of the animals collected during both summer and winter months were veliger stages. Laboratory observations showed that adult *A. gaudichaudi* swim in a circular course with the aperture pointed towards the water surface; they often rest at the surface. *A. gaudichaudi* feeds on euthecosomatous and gymnosomatous pteropods. Cannibalism is also common.

The family Atlantidae, which comprises approximately 15 species, are the only heteropods that can completely retract into their shells. The shells are planispiral, with a thin keel surrounding the teleoconch. Due to the fragility of their shells, they are not collected easily intact. As a consequence, species descriptions are often incomplete and most identifications dubious so that the taxonomy of atlantids remains confused, their distribution uncertain and their biology poorly understood.

Tesch (1949) reviewed the taxonomy and distribution of atlantids and synonymized many of the nominal species. Richter (1974) examined the taxonomy of atlantids from the Indian Ocean and described several new species. Van der Spoel (1976) reviewed the taxonomy and distribution of heteropods from the world's oceans. In the past, descriptions of the minute details needed for species differentiation, revealed under the light microscope, have been inadequate (Pilkington, 1970). Scanning electron microscopy (SEM) has proven to be an essential tool in the study of atlantid shell and radula morphology (Thiriot-Quievreux, 1973; Richter, 1974).

Shell morphology alone is inadequate to differentiate between closely related species. Species descriptions must deal with a range of characters such as morphology of the

live animal, shell surface sculpture (well revealed by SEM), veliger shell morphology, shape of the operculum and the eyes, and radula tooth morphology. Richter (1974) used many of these characters in separating Indian Ocean species. Although Tesch (1949) emphatically stated that differences in radula tooth morphology only showed generic differences, this is not necessarily the case since Richter (1961, 1986, 1987) has shown that some atlantid species can be separated by differences in radula morphology. Tokioka (1961) also showed that some species can be distinguished by the shape of operculum.

There are few records of atlantids from Australian waters. Smith (1888) reported *Atlanta gaudichaudi* Souleyet, 1852 from the Torres Strait and *A. peroni* Lesueur, 1817 'near' Cape York during the Challenger Expedition of 1873-1876. Cotton (1932) recorded *A. rosea* Smith, 1888 (= *A. peroni*) from dredged material off south Australia. From material collected during the Siboga and Dana Expeditions, Tesch (1906, 1949) reported *A. gaudichaudi* from the Torres Strait and noted that this atlantid was predominantly found in the Indo-Pacific. Dakin and Colefax (1940) also commented that *Atlanta* spp. were common off the coast of New South Wales. The only detailed study on heteropods from Australian waters was conducted by Russell and Colman (1935) as a seasonal study

of *A. peroni* in the waters of the northern Great Barrier Reef during the Great Barrier Reef Expedition of 1928 to 1929. Tesch (1949) found *A. turriculata* d'Orbigny, 1836 off the coast of New South Wales. Van der Spoel (1976) reported *A. inclinata* Souleyet, 1852 off northern Queensland and *A. lesueuri* Souleyet, 1852 and *A. helicinoides* Souleyet, 1852 from northwestern Australia. Ralph (1957) did not include atlantids in her review of the heteropods from New Zealand.

The global distribution and the taxonomy of *Atlanta gaudichaudi* is uncertain as this species is easily confused with *A. peroni* and *A. plana* Richter, 1972 (Tesch, 1949; Thiriou-Quiévreux, 1973; Richter, 1974). Jamieson and Newman (1989), in their study of the sperm ultrastructure of *A. gaudichaudi*, used specimens from Heron Island, thus extending the known range of this species in Australian waters southward from the Torres Strait to southern Queensland.

Swimming behaviour of atlantids has been briefly described by Tesch (1949), Wilbur and Yonge (1964), and Land (1982). Lalli and Gilmer (1989) reviewed the literature on atlantid biology and included some personal *in situ* observations on these animals. Observations of atlantids feeding on pteropods and gastropod larvae were reviewed by Thiriou-Quiévreux (1973), Richter (1982), and Lalli and Gilmer (1989). Anatomical and histological studies of the digestive and reproductive systems were made by Martoja and Thiriou-Quiévreux (1975a, b) and Thiriou-Quiévreux and Martoja (1976). Organogenesis of the veligers of six atlantid species (not including *A. gaudichaudi*) have been documented in detail by Thiriou-Quiévreux (1969). Pilkington (1970) also documented the metamorphosis of an unidentified atlantid from New Zealand waters.

This paper details the morphology of *Atlanta gaudichaudi*, collected within waters of the Great Barrier Reef (hereafter GBR) and provides for the first time descriptions of the live animal, veliger and radula for an Australian atlantid species. Data on seasonal distribution within the northern and southern GBR are presented, together with laboratory observations on swimming behaviour, feeding and prey selection, and the metamorphosis of late veliger larvae.

MATERIALS AND METHODS

Plankton collections were made from surface waters around Lizard Island (northern GBR, 14°14'S, 145°27'E) and Heron Island (southern GBR, 23°27'S, 151°55'E) (Fig. 1) from December 1985 to February 1988. Each sampling trip was of two to three weeks duration. Approximately 200 samples (20 samples per trip) were collected from the upper 1.0 to 1.5 m of the water column during daylight hours in summer and winter months. The bottom depth varied between 10-30 m at both locations. Quantitative plankton nets (with Oceanic Model No. 2030 flowmeters), 300 mm mouth diameter, with 0.2 mm mesh size were used. Tows were made once daily for 10 min duration, this being brief enough to minimize shell damage and ensure animals were alive after collection. All nets were equipped with soft cod ends (made of canvas) to further reduce shell damage. Immediately after collection, specimens were sexed and measured with an ocular micrometer on a



Fig. 1. Map of Queensland, Australia showing the locations of Lizard and Heron islands on the Great Barrier Reef.

Wild M5 dissecting microscope (accuracy to 0.01 mm). Size was expressed as the maximal shell diameter, excluding the keel which is often broken after collection. Animals were subsequently preserved in 70% EtOH.

The numbers of *Atlanta gaudichaudi* present in each sample (=abundance) were expressed as number of animals/m³; the volume filtered being calculated from flowmeter readings, net diameter and distance towed. Comparisons of mean abundances between sampling periods were tested by One-Way ANOVA and Student's t-tests (tested at the 95% confidence level) on log transformed data (Table 1). The relative percent of each life history stage was calculated for each sampling trip from the actual number of animals measured.

Individuals were retained in the laboratory in aerated 250 ml containers for feeding observations, and 5 l containers for swimming behavioural studies. Seawater was changed twice daily. In feeding trials, zooplankton prey was introduced individually and in groups to each container. Zooplankton was removed within four hours if it was not consumed by an atlantid. Live animals from plankton samples with food items in their oesophagus were induced to disgorge their gut contents by the addition of a drop of 5% glutaraldehyde.

Alcohol preserved specimens for scanning electron microscopy were sonically cleaned for less than 10 sec, placed

on double-sided sticky tape on aluminum stubs and gold coated. Radulae were dissected from preserved animals with the buccal tissue intact, soaked in 10% KOH for 24-48 hours, then rinsed in distilled water. Due to the small size of the radulae (less than 0.3 mm in length) each radula was manipulated by fine insect pins and placed in a drop of distilled water on a stub covered with double-sided sticky tape. As the water evaporated, the radula was positioned on the stub and then coated. Photomicrographs were taken with 400 ASA Pan-X black and white film, at 20 KV with a Philips 505 scanning electron microscope.

RESULTS AND OBSERVATIONS

SPECIES DESCRIPTION

Atlanta gaudichaudi Souleyet, 1852 (Figs. 2-4)
(for synonymies see van der Spoel, 1976)

The shell of this species is smooth and devoid of surface sculpture except for one fine spiral line on the outer edge of the apical whorls. The shell has four whorls, the outer whorl is relatively large and expands into an inflated aperture. The three apical whorls gradually increase in width. The spire is relatively low, not extending past the height of the body whorl. The keel does not penetrate between the outer whorl. The shell is transparent and colourless except for a faint red-brown colour at the base of the keel. Adults range from 1.00 to

2.25 mm in diameter. Sexually mature males are distinguished by a bilobed penis on the right side of the neck and, if present, a black and white oval spermatophore. Females are distinguished by the presence of developing ova, seen clearly through the shell in the apical whorls.

The eye tentacles are equal in length and do not extend beyond the distal end of the proboscis. The body of the animal is colourless. The eyes are of Richter's (1974) Type b, having a transverse slit present in the distal portion of the pigmented retina. The operculum is also Richter's (1974) Type b, micro-oligogyre, or having only a small gyre on its periphery. The radula of *Atlanta gaudichaudi* is typically taenioglossan in morphology, with a formula of 2-1-1-1-2 (Figs. 3C, D).

Veligers ranged in shell diameter from 0.04 to 0.40 mm. The shell is smooth and colourless, with a single spiral line on the outer edge of each whorl (Figs. 3A, B). In the smallest veligers, the velum is divided into four lobes, becoming six lobes prior to metamorphosis (Fig. 4B). The lobes, prior to metamorphosis, are only slightly wider than the shell diameter. The shell and body are colourless except for a red-brown or yellow-orange colouration of the digestive gland. Late veligers have well developed eyes with a clear lens and black retina, eye tentacles, and a small fin with a sucker. The aperture of the shell is indented and formation of a small keel can be seen. Juveniles ranged from 0.50 to 0.90 mm in shell diameter. Young males are distinguished by the presence of two small penial "buds" on the right side of the neck.

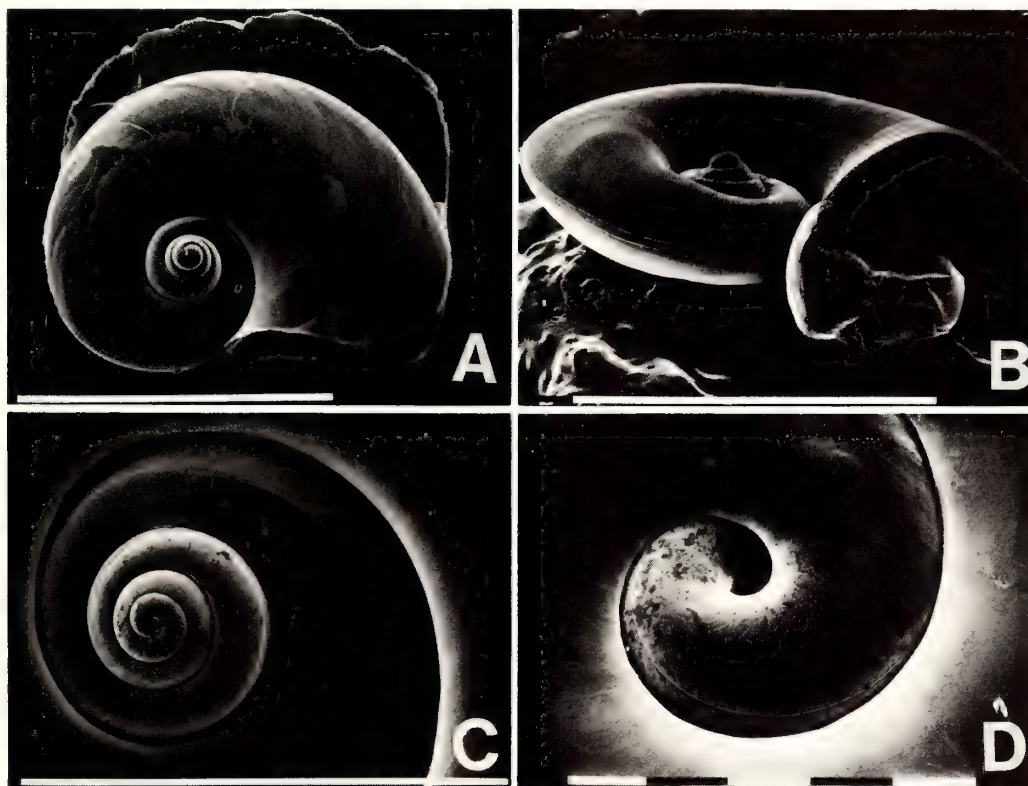


Fig. 2. Scanning electron micrographs of *Atlanta gaudichaudi*: A, dorsal view; B, aperture view; C, close-up of apical whorls; D, umbilicus (A-C, scale bar = 1 mm; D, scale bar = 0.1 mm).

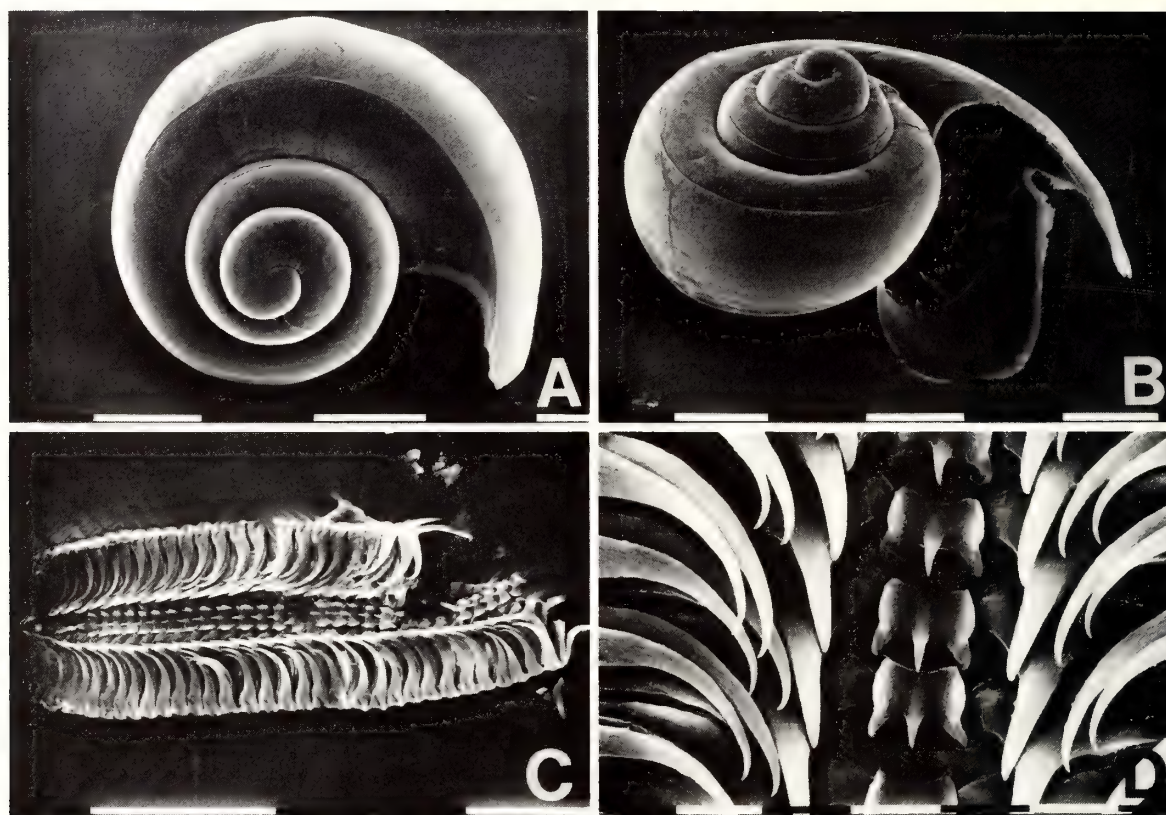


Fig. 3. Scanning electron micrographs of *Atlanta gaudichaudi*: A, late veliger, dorsal view; B, late veliger, aperture view; C, whole radula; D, median and lateral radular teeth (A, C, scale bar= 0.1 mm; D, scale bar= 10 μm).

REMARKS

Atlanta gaudichaudi can be easily confused with *A. peroni* and *A. plana* which are similar in shell morphology when examined by only light microscopy (Table 2). *A. gaudichaudi* mainly differs from *A. peroni* in maximum shell diameter and morphology of the apical whorls. *A. gaudichaudi* reaches a maximal diameter of 5 mm (twice the diameter recorded in the present study) whereas *A. peroni* can reach up to 11 mm (van der Spoel, 1976). Richter (1974), using SEM has shown that the apical whorls of *A. peroni* from the Indian Ocean have a distinct layer of callus running along the suture and a spiral line on the centre. In contrast, the apical whorls of *A. gaudichaudi* only have a faint spiral line on the outer margin. The shell aperture is larger in *A. gaudichaudi* than in *A. peroni*. The keel only penetrates between the outer whorl in large specimens of *A. gaudichaudi* whereas the keel strongly penetrates between the outer whorl of *A. peroni* (Tesch, 1949; van der Spoel, 1976).

Differences are seen in the morphology of the lateral radular teeth of *Atlanta gaudichaudi* from different oceans as

specimens from Lizard and Heron islands do not possess a secondary spine on the inner margin as shown by Richter (1961) for *A. gaudichaudi*.

Atlanta plana is also similar in shell shape to *A. gaudichaudi*. The most important difference is that the operculum of *A. plana* has fine hooks or spines on its central gyre (Richter, 1974), whereas the operculum of *A. gaudichaudi* does not have spines. In adults, the eyes are Type a (large pigmented retina and cuboidal shape) in *A. plana* compared with Type b in *A. gaudichaudi* (Richter, 1974). Adult *A. plana* can also be distinguished by the purple-violet colour of the apical whorls (van der Spoel, 1976). Veligers of *A. plana* were rarely collected in this study but they could be distinguished from those of *A. gaudichaudi* by their darker purple colour and the numerous fine spiral striae on the shell surface.

SEASONAL DISTRIBUTION

Atlanta gaudichaudi was the most common species encountered, being found in all seasons at both Lizard and Heron islands. *A. lesueuri*, *A. plana*, *A. helicoides*, and *A.*

Table 1. T-Test of Log mean abundance of *Atlanta gaudichaudi* from Lizard and Heron islands (*significant at 95% confidence level).

Heron vs. Lizard	Heron Summer vs. Lizard Summer	Heron Winter vs. Lizard Summer	Heron Summer vs. Heron Winter	Lizard Summer vs. Lizard Winter
0.197	6.398*	6.679*	9.040*	3.885*

Table 2. Comparison of the morphological features of *Atlanta gaudichaudi*, *A. peroni* and *A. plana* (from Richter, 1974; van der Spoel, 1976).

Species	Maximum Diameter (mm)	Number of Whorls	Apical Whorls	Penetration of Keel	Eyes	Operculum	Colour
<i>A. gaudichaudi</i>	5	4	smooth, 1 spiral line	absent	Type b	Type b	base of keel brown/red
<i>A. peroni</i> Lesueur, 1817	11	5	smooth, 1 spiral line, layer of callus	present	Type b	Type b	base of keel brown
<i>A. plana</i> Richter, 1972	5	5	2 spiral lines	absent	Type a	Type b spines on gyre	apical whorls purple/pink

fusca Souleyet, 1852 were rarely encountered in either summer and winter months at Lizard or Heron Islands. *A. turriculata* and *Oxygyrus keraudreni* (Lesueur, 1817) were rarely collected and only at Lizard Island.

Overall, the mean abundance of *Atlanta gaudichaudi* varied between 0.2 to 5.2 animals/m³ at Lizard Island and between 2.2 to 17.3 animals/m³ at Heron Island. Abundances showed the greatest seasonal variation at Heron Island, where higher numbers were encountered in summer than winter months (Fig. 5). At Lizard Island greater numbers were found in winter, and the range of mean abundances was not as broad as at Heron Island.

Comparisons of the mean abundances for combined summer versus winter samples (Fig. 6, Table 1) indicated high variability in the number of animals collected. Although, there was no significant difference in the overall abundance of *Atlanta gaudichaudi* between Lizard and Heron Islands (all sampling trips combined) there were highly significant seasonal differences both between and within sampling areas (95% confidence level).

The percentages of each life history stage present in the waters off Lizard and Heron islands showed an overall similarity in population structure (Figs. 7, 8). The majority of individuals collected were veliger stages in both sampling

seasons. Figure 7 shows that juvenile, male and female components each represented less than 10% of individuals from Lizard Island. At Heron Island, juveniles accounted for up to 20% (Winter 1986) and males and females less than 10% of individuals collected (Fig. 8). There are no clear seasonal differences in the ratios of the veliger, juvenile or adult stages for either Lizard or Heron islands. Usually, juveniles accounted for only a slightly larger ratio of animals compared to adults. A relatively higher ratio of juveniles were found in winter compared to summer for Heron Island. Generally there were more females than males at both sampling locations, although the actual numbers of adults were too low to permit statistical analyses.

BIOLOGY

Observations were made on the swimming behaviour of *Atlanta gaudichaudi* in the laboratory. The maximum period individuals could be kept alive was seven days. When swimming, individuals move in a circular course and the motion is jerky and sporadic (Fig. 9A). The shell aperture is directed towards the water surface and the foot is directed dorsally during swimming. The well developed foot muscles move the fin from side to side in a flapping motion which is counteracted

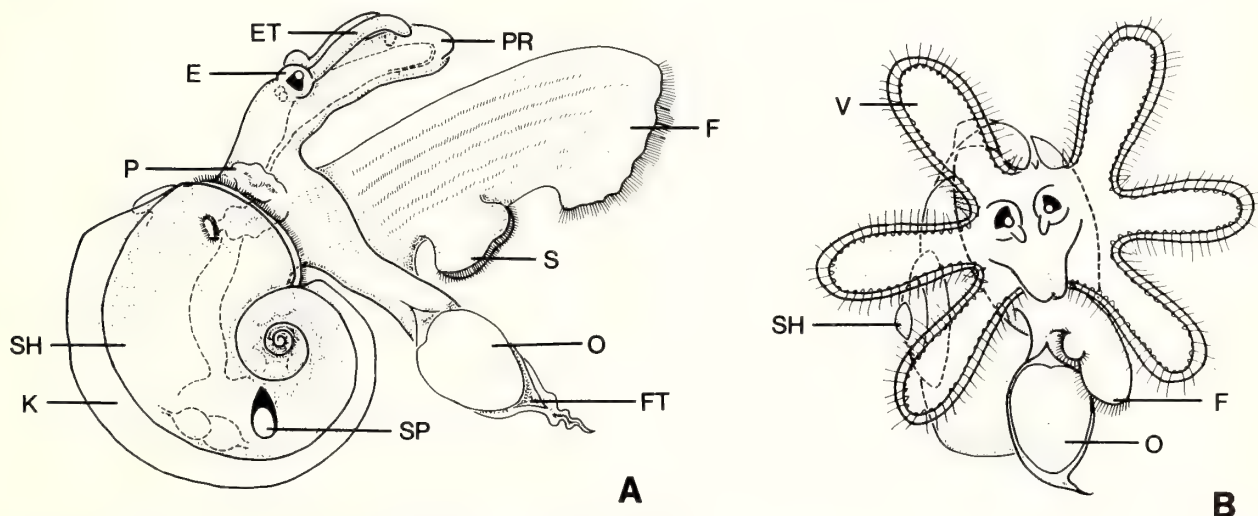


Fig. 4. Morphological features of *Atlanta gaudichaudi*: A, male with body extended (after Jamieson and Newman, 1989); B, late veliger, prior to metamorphosis (E, eye; ET, eye tentacle; F, fin; FT, foot; K, keel; O, operculum; P, penis; PR, proboscis; S, sucker; SH, shell; SP, spermatophore; V, velum).

by the swinging of the shell. Upward swimming always resulted in the animal hitting the water surface with its proboscis. Frequently, animals were seen to 'attach' to the water surface with their proboscis for a few minutes (Fig. 9B). When sinking, the head is tilted backwards, the fin and foot held vertically above. Sinking rates ranged from 0.5 cm/sec for juveniles, to 1.7 cm/sec for adults. Some were observed to slow their sinking rate and remain suspended in the water column. When currents were generated in the container, individuals were seen suspended in a resting position with the aperture directed toward the water surface and the fin and foot held motionless above (Fig. 9C). When the currents subsided, all animals sank to the bottom. Once on the bottom, they spent a few minutes cleaning their shell, keel and foot with their proboscis. Most individuals, when disturbed with a fine probe, appeared to have mucus 'threads' attached to their aperture or foot. Suspended animals were also observed on a few occasions hanging to these threads in the water column.

Atlanta gaudichaudi capture euthecosomatous pteropods by grabbing the prey's shell with their fin and fin sucker. The fin sucker attaches to the shell, the fin wraps around it and the proboscis extends into the prey's aperture (Fig. 10). Slowly, the prey's wings are drawn out and into the atlantid's oesophagus. The atlantid does not let go of its prey until the pteropod's wings and viscera are consumed. In the case of creseid pteropods [*Creseis acicula* (Rang, 1828), *C. virgula* (Rang, 1838) and *C. chierchiae* (Boas, 1886)], the columellar muscles attaching the body to the shell were severed by the radula of the atlantid. Animals were also observed feeding on limacinid pteropods [*Limacina trochiformis* (d'Orbigny, 1836) and *L. inflata* (d'Orbigny, 1836)]. The atlantid's sucker grasped the coiled shell while the proboscis removed the wings and viscera. Capture of food took from one minute to one hour, while digestion of ingested food, observed as it moved into the stomach, took up to 6 hours. On one occasion, *A. gaudichaudi* was seen to consume a

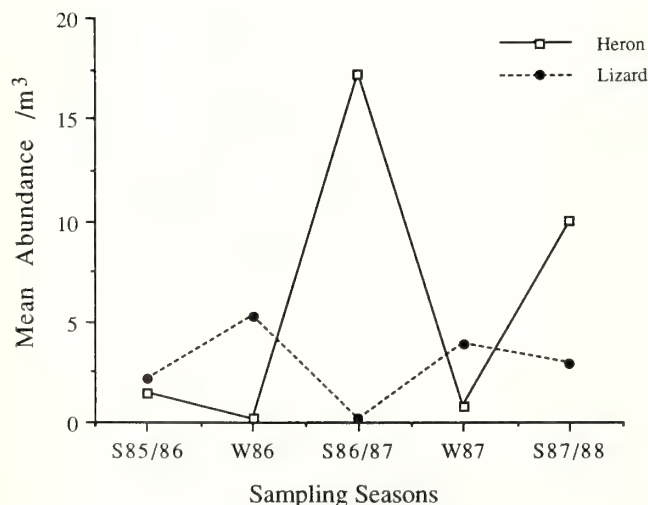


Fig. 5. Mean seasonal abundance of *Atlanta gaudichaudi* at Lizard and Heron islands, 1985-1988.

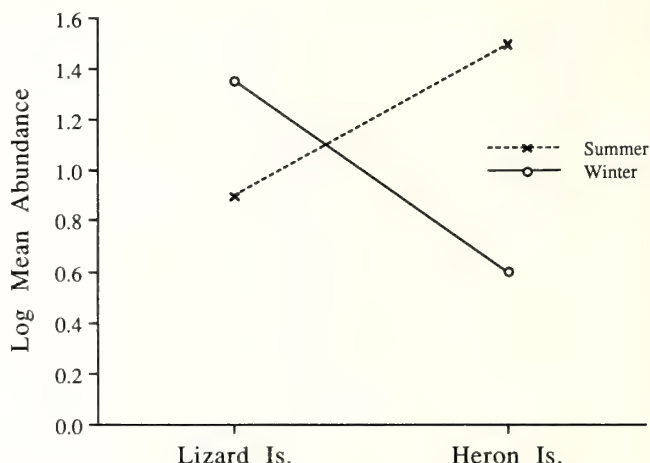


Fig. 6. Summer and winter averages in the Log mean abundance of *Atlanta gaudichaudi* from Lizard and Heron islands, 1985-1988.

gymnosomatous pteropod (*Pneumodermopsis spoeli* Newman and Greenwood, 1988).

Other zooplankters offered to captive *Atlanta gaudichaudi* included copepods, prosobranch and bivalve veligers, crab larvae, mysids, ostracods, chaetognaths, salps, medusae, larval fish and radiolarians. None of these zooplankters were attacked or consumed by *A. gaudichaudi*. Cannibalism was frequently observed on smaller sized atlantids and veligers. Direct observations on food capture showed that *A. gaudichaudi* most frequently preyed on euthecosomatous pteropods.

Examination of food material from the oesophagus of freshly caught *Atlanta gaudichaudi* revealed the presence of statocysts of euthecosomatous pteropods (as many as five pairs) and pairs of atlantid eyes. Identifiable regurgitated stomach contents also contained parts of limacinid and creseid pteropods and atlantids.

Late stage veligers swam with the aid of their lobes as well as the small fin. On several occasions, the metamorphic loss of the velar lobes of late veligers was observed under the dissecting microscope. At metamorphosis the lobes could be clearly seen being consumed by the radula. Consumption of the lobes took up to 4.0 hr, with removal of both sides of the velum at the same time. Late veligers were never seen with an asymmetrical velum as described by Pilkington (1970).

DISCUSSION

Atlanta gaudichaudi is the most common species of heteropod found within the waters of the Great Barrier Reef and is more abundant in the southern region (subtropical waters), especially in summer months. In the Atlantic Ocean, *A. gaudichaudi* has a wide latitudinal range of distribution from 60°N to 25°S (van der Spoel, 1976).

Atlanta peroni was not found at either Heron or Lizard islands during this study and it is highly probable that reports of this species from off Low Isles (Russell and Colman, 1935)

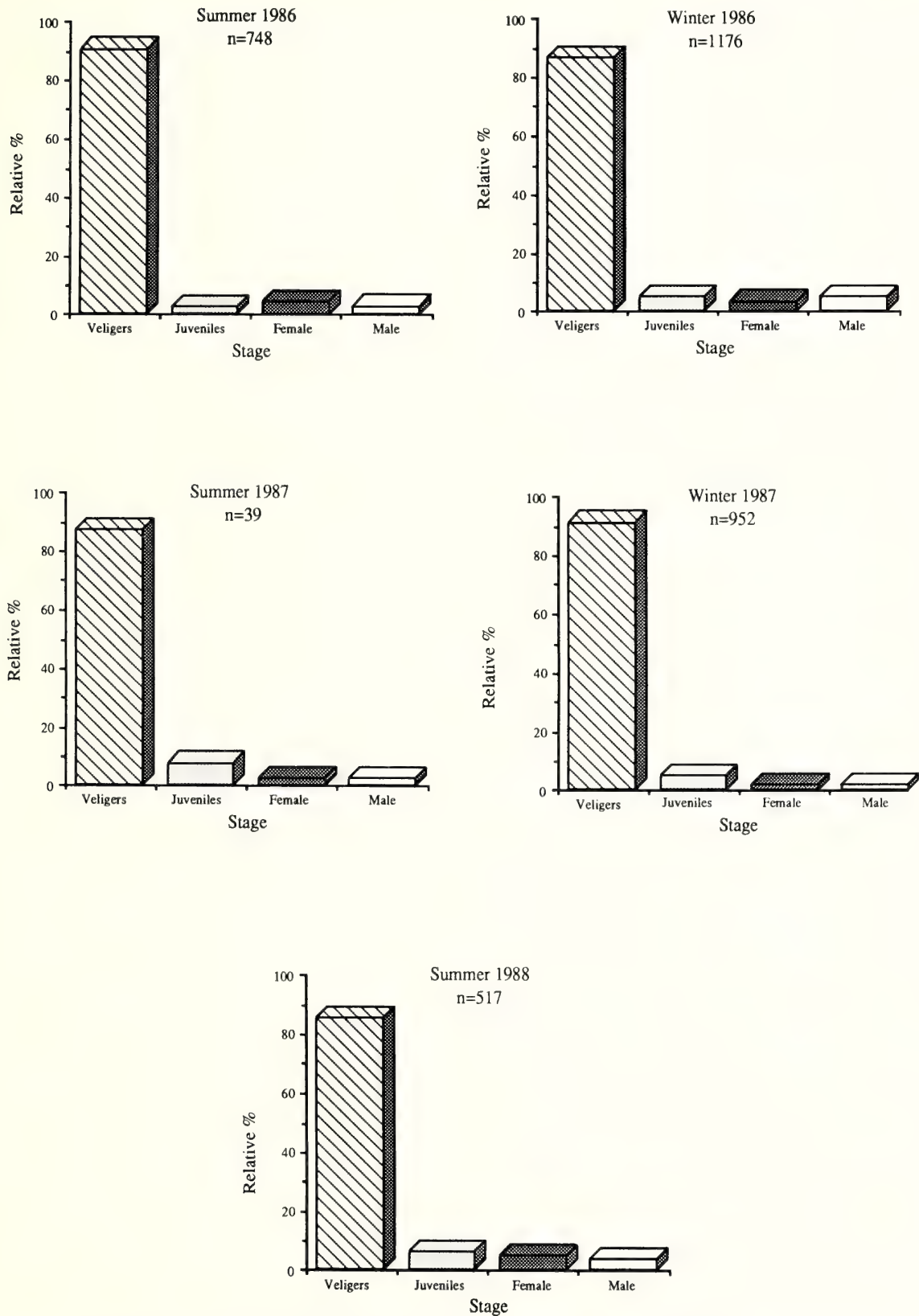


Fig. 7. Relative percent of each life history stage of *Atlanta gaudichaudi* from Lizard Island, 1986-1988.

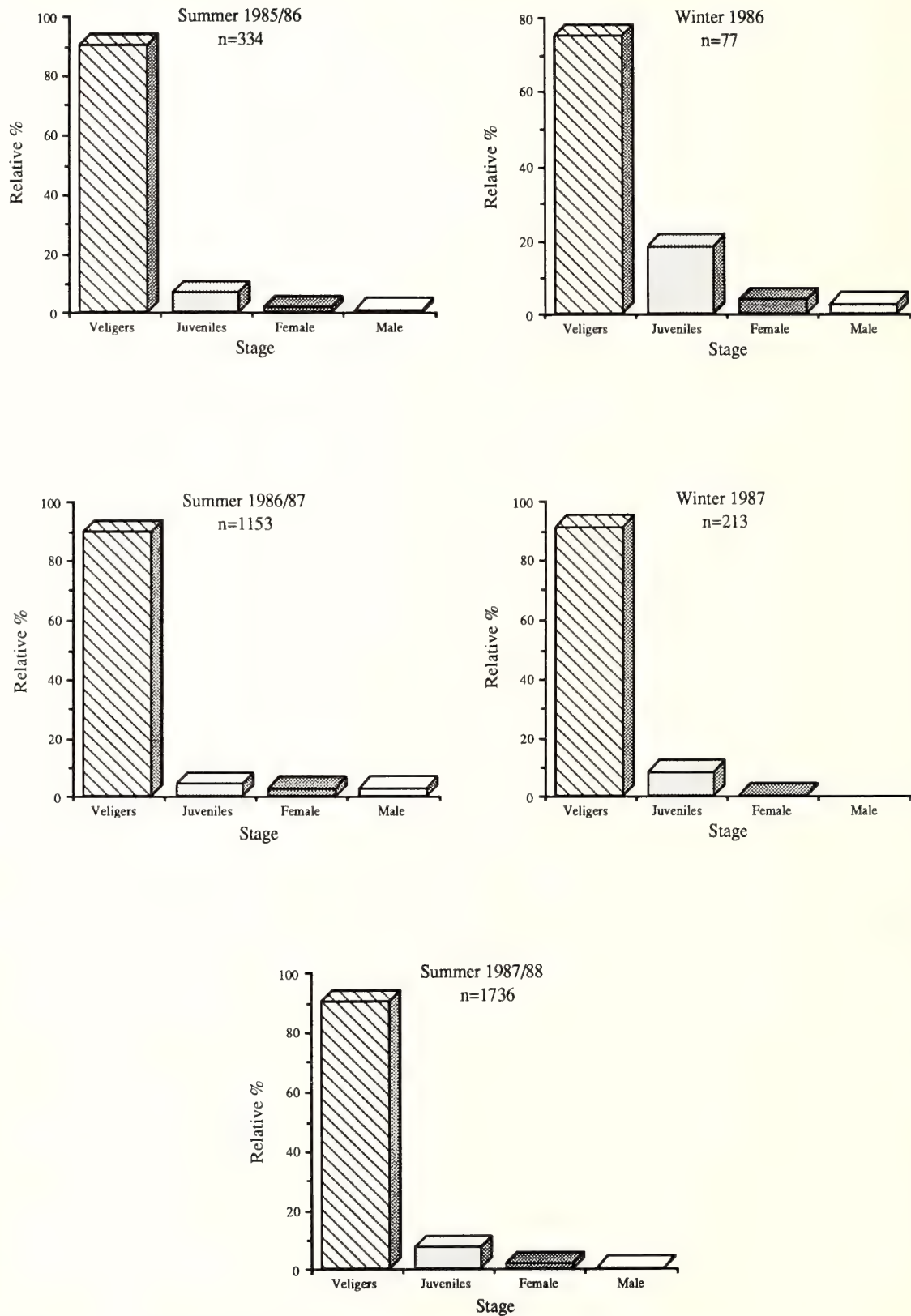


Fig. 8. Relative percent of each life history stage of *Atlanta gaudichaudi* from Heron Island, 1985-1988.

are erroneous. Support for this contention lies in the facts that: *A. peroni* is easily confused with *A. gaudichaudi* due to similarities in shell shape; "*A. peroni*" is the only species collected by these authors in the GBR region; their specimens were all relatively small in size (2.5 to 4.5 mm); they found no apparent seasonal change in abundance of "*A. peroni*" within the GBR Lagoon (also found for *A. gaudichaudi* at Lizard Island in the present study); smaller individuals were collected from July to September and larger animals were found from October to January (these authors did not account for veligers of "*A. peroni*"). Similarly a higher ratio of juveniles to adults of *A. gaudichaudi* was found at Heron Island during winter months in the present study.

The majority of individuals of *Atlanta gaudichaudi* were in the veliger stage at both sampling locations. This could account for this atlantid being overlooked or considered seemingly rare in past studies. The number of individuals varied greatly between sampling trips, but the relative percentage of veligers remained high. Richter (1968) also found high numbers of atlantid veligers within surface waters. In addition to natural mortality, the high number of veligers could have been due to bias in sampling due to the small mesh size used, or to differences in diurnal vertical migration of the life history stages. Richter (1973) showed that heteropod larvae have a more extensive diurnal migration than the adults and concluded that a nocturnal maximum in surface waters is due to the vertical migration of larvae. Nocturnal samples were not collected during the present study. Frontier (1973) showed that veligers of *A. gaudichaudi* from the coast of Madagascar had a neritic distribution while the adults were oceanic and results from this study indicate a similar distributional pattern.

Heteropods are known to swim with the aperture of the shell pointing towards the water surface and the foot directed dorsally. The eyes scan the water below in a slow smooth motion for stationary prey that reflect the light from above (Land, 1982). Tesch (1949) described swimming as a continual jerky movement, keeping the animal afloat. Atlantids undulate their fin like a sculling blade (Wilbur and Yonge, 1964). My observations indicate that atlantids use their fin for propulsion and their foot and shell as stabilizers. The flat surfaces of the shell, especially the thin keel, counteracts the motion of the fin. Atlantids also appear to rest at the water surface and remain suspended in water currents. It could also be possible that they attach themselves with their proboscis (radula) to other floating surfaces, such as abandoned euthecosomatous pteropod feeding webs or that they use mucus threads as a parachute. Lalli and Gilmer (1989, Fig. 11) observed an unidentified atlantid, *in situ*, suspended from mucus threads originating from the foot.

Literature on the feeding biology of atlantids is scarce. Richter (1968) observed *Atlanta peroni* feeding on euthecosomatous pteropods. Thiriot-Quievreux (1969, 1973) found; atlantids fed on creseid pteropods, gastropod veligers and atlantids; digestion was completed in 24 hr; and the prey is consumed one at a time. My observations showed that individual *A. gaudichaudi* often had several pairs of creseid statocysts or atlantid eyes in the oesophagus at any one time, indicating that they feed on more than one prey at a time.

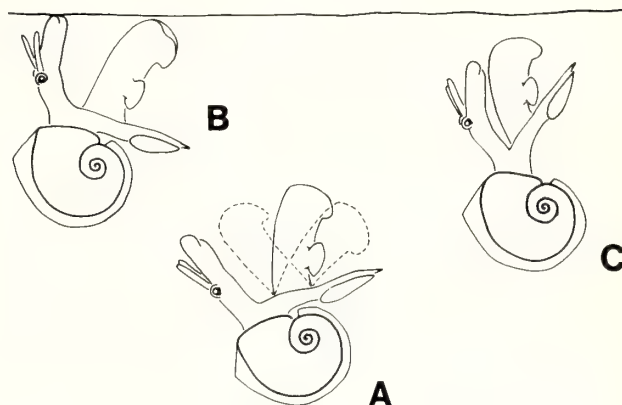


Fig. 9. Diagram of observed behaviour of *Atlanta gaudichaudi*: A, swimming; B, resting at surface; C, sinking.

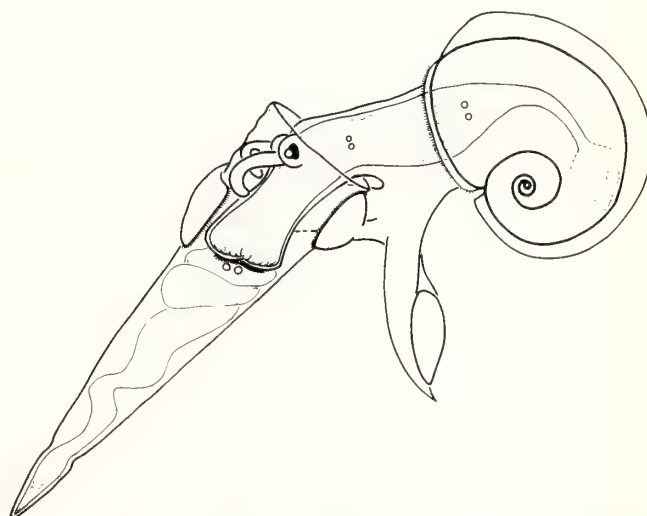


Fig. 10. Diagram of *Atlanta gaudichaudi* consuming the pteropod, *Creseis chierchiaie*.

Richter (1982) examined the stomach contents of *Oxygyrus keraudreni* and showed that this species feeds mainly on gastropods (pteropods and heteropods) but also copepods and chaetognaths. My results indicate that *A. gaudichaudi* prefers to feed on creseid and limacinid pteropods under laboratory conditions. It appears from the limited literature that atlantids are selective predators, feeding mainly on other planktonic gastropods. These transparent carnivorous zooplankters probably remain suspended, and motionless at or near the water surface when they are not feeding. Swimming would only be employed for escaping predators and for active seizure of prey.

ACKNOWLEDGMENTS

Research was funded by the Australian Federation of University Women-Queensland Branch, the Hawaiian Malacological Society, the Australian Museum, the Coral Reef Society and the Zoology Department University of Queensland. Special thanks are given to the Directors and staff of the Heron and Lizard Island Research Stations, Dr.

J. G. Greenwood for his advice and comments, Dr. S. van der Spoel for his critical review, Mr. P. Davie for his comments, the Electron Microscope Centre University of Queensland, Ms. M. Preker for assistance in the field and Mr. A. Flowers for technical support.

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Date of manuscript acceptance: 3 November 1989



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Seed, R. 1980. Shell growth and form in the Bivalvia. In: *Skeletal Growth of Aquatic Organisms*, D.C. Rhoads and R.A. Lutz, eds. pp. 23-67. Plenum Press, New York.

Vail, V.A. 1977. Comparative reproductive anatomy of 3 viviparid gastropods. *Malacologia* 16(2):519-540.

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AMERICAN MALACOLOGICAL BULLETIN

VOLUME 8 NUMBER 2

APRIL 1991

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Cover. *Io fluvialis* (Say, 1825) is the logo of the American Malacological Union and the subject of a paper by Ahlstedt in this issue.

THE AMERICAN MALACOLOGICAL BULLETIN is the official journal publication of the American Malacological Union.

AMER. MALAC. BULL. 8(2)
April 1991

GENETIC STUDIES OF ASIATIC CLAMS, *CORBICULA*, IN THAILAND: ALLOZYMES OF 21 NOMINAL SPECIES ARE IDENTICAL

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ABSTRACT

Freshwater clams of the genus *Corbicula*, collected from 40 sites up to 1500 km apart in Thailand, and representing 21 nominal species, show no significant geographic variation at 24 electrophoretically detected allozyme loci and are most probably all referable to the widespread Asian species, *C. fluminea* (Müller, 1774).

Thai *Corbicula* have very little genetic variability: mean number of alleles per locus ($\bar{A}$) was very low: no variation ($\bar{A} = 1.0$) was detected in 30% of the samples, in the remainder, $\bar{A} \leq 1.1$; mean percentage of loci polymorphic in each sample, $\bar{P} = 4.59\%$ (range: 0.0-12.5%); mean individual heterozygosity, $\bar{H} = 0.011$ (range: 0.000-0.025) with one outlier where $\bar{H} = 0.058$). The low level of population variability and very low individual heterozygosity suggest that most of the *Corbicula* in Thailand are facultative self-fertilizers. The small amount of genic diversity detected, and the observed genotype frequencies, are apparently maintained by limited outcrossing, at random with respect to shell phenotype, internal shell color and allozyme genotype.

Eighty-eight percent of the samples, including one referable to *Corbicula fluminea*, cluster at Nei's genetic distance values of $D < 0.01$. Only five samples from northeast Thailand stand slightly apart from the others. These very high genetic similarities, coupled with a lack of significant anatomical variation, provide no support for the recognition of more than one species in our samples. Twenty nominal Thai species are synonymized accordingly with *C. fluminea*; another seven nominal species are candidates for synonymy.

Freshwater clams of the genus *Corbicula* (Bivalvia: Corbiculidae) occur throughout Asia, eastern Europe and Africa. One species, *C. fluminea* (Müller, 1774), is the commonest freshwater bivalve in Asia and has recently been introduced to western Europe, North and South America (Morton, 1986). In the United States it spread rapidly and, as a major economic pest, it has been the subject of considerable

study and two international conferences (Britton, 1979, 1986). This research has shown that *C. fluminea* is quite variable in shell size, shape, sculpture and color: the same conchological characteristics upon which most *Corbicula* species have been defined. Morton (1979, 1986) reexamined such conchological variation in the numerous nominal species of *Corbicula* from China and Japan and found that such characters were

taxonomically unreliable. He found that, when morphological variation was considered together with data on ecology, physiology, demography and reproductive behavior, only two biological species could be recognized in east Asia: freshwater *C. fluminea*, and estuarine *C. fluminalis* (Müller, 1774) (Morton, 1986). He suggested that most of the other nominal Asian taxa are synonyms of these two species. This paper examines this hypothesis as it applies to the nominal species of *Corbicula* in Thailand.

Most of the original species descriptions of Asian *Corbicula* are due to Prime (1864) and Prashad (1930, and references therein). Subsequently, Brandt (1974) cataloged the molluscs of Thailand and recognized 28 species of *Corbicula*, five of them new. These systematists worked almost entirely with conchological characters in defining their species and ignored geographic and ontogenetic variation and other biological attributes. The senior author has recently described variation in *Corbicula* representing 22 nominal species from 40 localities throughout Thailand (Kijiviriya, 1990). In agreement with earlier reports, she found considerable conchological variation among clams referable to the different species recognized by Brandt (1974). She also found significant variation in shell size, shape, sculpture and color within and between allegedly conspecific populations. Gross anatomy and dissections of the siphon, digestive and reproductive systems, on the other hand, failed to reveal any taxonomically significant variation between any of the populations sampled (Kijiviriya, unpub. data). In this paper, we report on detectable genetic variation within and between these populations and conclude that a major taxonomic revision of Thai *Corbicula* is now required.

Genetic variation in *Corbicula* was studied by electrophoretic surveys of multilocus allozyme patterns. Allozymes have proven extremely useful in recent systematic studies of molluscs. Intrapopulation allozymic variation has been used to establish mating systems (Selander and Kaufman, 1973; McCracken and Selander, 1980; Selander and Whittam, 1983), to reveal the existence of sibling species (Davis, 1983; Staub *et al.*, 1990), to reveal cases of interspecific hybridization (Gould and Woodruff, 1986, 1987, 1990; Woodruff and Gould, 1987) and to study environmental effects on population structure (Koehn and Hilbish, 1987; Nevo, 1988). Studies of interpopulation allozymic variation have revealed the extent of geographic structuring and variation in widespread species (Gould and Woodruff, 1978; Grant and Utter, 1988) showing whether disjunct populations are or are not conspecific (Palmer *et al.*, 1990; Woodruff *et al.*, 1988), revealed the historical pattern of a species dispersal (Mulvey *et al.*, 1988; Woodruff *et al.*, 1986, 1988), and helped define the limits of semispecies in syngameons (Woodruff and Gould, 1980; Woodruff, 1989). The comparison of allozymes among nominal species has supported numerous taxonomic revisions and phylogenetic discussions (Davis *et al.*, 1981; Emberton, 1988; Woodruff and Solem, 1990; Woodruff *et al.*, 1987). Given the demonstrable utility of such data on allozymic variation, this approach was included in our study of the Thai *Corbicula*.

Three research groups have previously reported

studies on allozyme variation in *Corbicula*. Smith *et al.* (1979) found no detectable variation at 18 loci in five populations from across North America. In contrast, they found some variation at 12-17 loci in four Asian samples: the proportion of loci that were variable (P) in the Philippines was $P = 0.17-0.23$, in Hong Kong, $P = 0.25$, and in Japan, $P = 0.76$. The Asian clams were thus moderately to highly variable and the monomorphism of the introduced North American populations was attributed to genetic drift associated with the founder effect during a single colonization. McLeod and Sailstad (1980) then reported consistent (year-round) low levels of genetic variation at seven loci from one population in eastern U.S.A.: $P = 0.14$, and mean individual heterozygosity, $H = 0.005$. They concluded that their genetic observations supported the hypothesis that North American *Corbicula* are self-fertilizing. Subsequently, McLeod (1986) reported a study of variation in other populations from both eastern and western U.S.A. and again found little variation within samples. These earlier reports, especially that showing high genetic variability elsewhere in Asia, suggested that allozymic variation might be used in resolving the relationships among the *Corbicula* of Thailand.

MATERIALS AND METHODS

Clams were collected in 1985-1986 at 40 localities (Fig. 1, Appendix) from various provinces throughout Thailand. Most clams were collected from lotic habitats in the rivers or irrigation canals; exceptions were two lake localities (samples 1 and 23). Some clams were stored in liquid nitrogen immediately and hand-carried to the University of California, San Diego (UCSD) for allozymic screening and protocol development; others were maintained alive at Mahidol University and used for routine allozyme studies. Notes on clam density, shell morphology, and habitat type and water pH were recorded. Distributional and conchological criteria (Brandt, 1974) were used to identify 39 of the 40 samples to the species level. Voucher specimens from each sample were deposited in the Museum at the Center for Applied Malacology and Entomology, Mahidol University, Bangkok. Voucher specimen lot numbers are given in the Appendix.

ELECTROPHORESIS

Proteins from 30 animals from each sample were studied by electrophoretic analysis using horizontal starch gel slabs following the techniques of Selander *et al.* (1971). Prior to electrophoresis, individual clams were thawed and quickly homogenized in 0.1-0.2 ml distilled water. The whole-body homogenate was centrifuged at 4,500 g for 10 min and the supernatant was absorbed onto Whatman No. 1 filter paper wicks (3x9 mm) and inserted into 12% (w/v) horizontal starch gels. Electrostarch Lot 146 (Otto Hiller, Madison, Wisconsin) was used throughout this study. Clams from different samples were run on each gel to facilitate comparisons and sample 17 from the Loei River (northeast Thailand) was used as a control group. Three buffer systems were employed to resolve the 14 enzyme systems and 24 allozymes studied (Table 1).

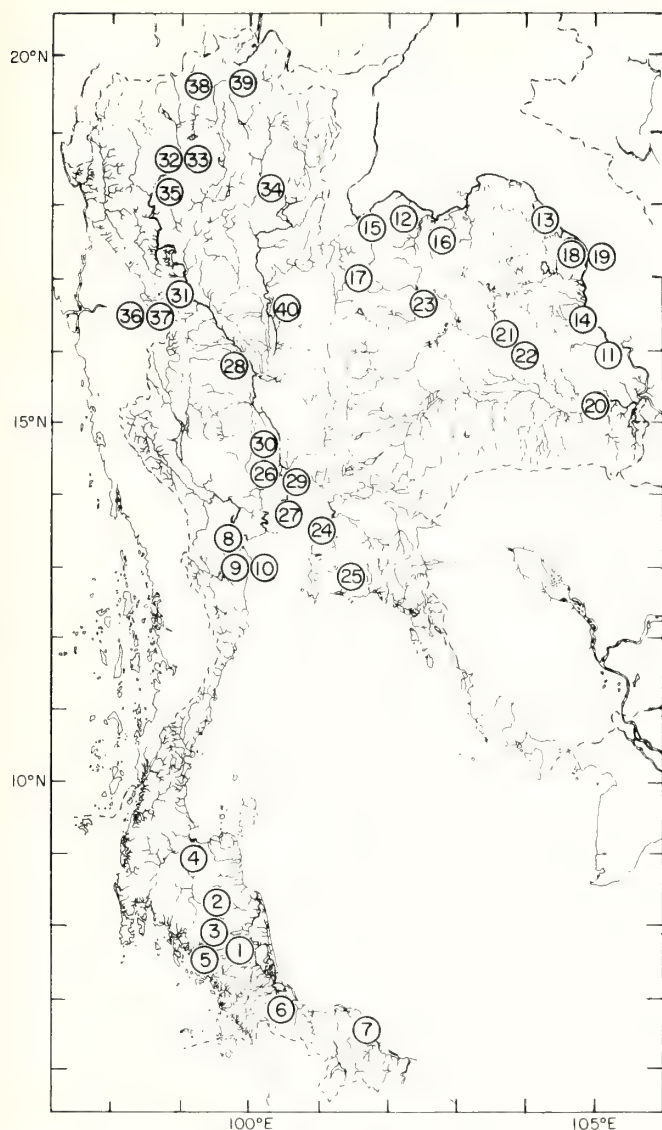


Fig. 1. Location of sample sites in Thailand.

Electrophoresis was carried out at constant voltage for 15 hr by which time a bromophenol blue marker dye had migrated 100-120 mm anodally. Following electrophoresis, each slab was cut into four or five slices and stained for a specific enzyme following standard methods (Murphy *et al.*, 1990). Isozymes were numbered, and allozymes were assigned superscripts (*a*, *b* or *c*) in order of decreasing anodal mobility. Commonly used enzyme abbreviations are typeset in italics to indicate the presumed genetic locus.

STATISTICAL ANALYSES

Data consisting of multilocus genotypes for individual clams scorable at all 24 loci were analyzed using the BIOSYS-1 computer program (Swofford and Selander, 1981). A locus was considered polymorphic if more than one allele was detected. Mean heterozygosity per individual ($\bar{H}$) was

estimated by direct count. Chi-square and Fisher exact tests were used to compare the frequency of individual genotypes with Hardy-Weinberg expectations for a panmictic population. Population structure was examined using fixation indices or *F* - statistics calculated for each locus and sample (Wright, 1978). Nei's (1978) unbiased genetic distance coefficients (*D*) and Rogers' (1972) genetic similarity coefficients (*S*) were calculated and clustered using the unweighted pair group averaging method (UPGMA) for the construction of phenograms and Wagner trees.

RESULTS

We obtained genetically interpretable results for 24 loci per sample. Descriptions of banding patterns and their interpretations are provided by Kijviriya (1990). Allozymic variation in 40 samples from throughout Thailand is summarized in Table 2.

The mean number of alleles per locus ($\bar{A}$) was very low: no variation ($\bar{A} = 1.0$) was detected in 12 of the 40 samples (30%) and in the remaining 28 samples, $\bar{A} \leq 1.1$. The mean percentage of loci that were polymorphic in each

Table 1. Electrophoretic buffers giving optimal resolution of 24 allozymic loci in *Corbicula* sp.

Allozyme (E.C. No.)	Abbreviation	Buffer*
Alkaline phosphatase (3.1.3.1)	<i>Alp</i>	TC 6.0
Aspartate aminotransferase (2.6.1.1)	<i>Aat-1</i>	TC 6.0
	<i>Aat-2</i>	TC 6.0
Esterase (alpha-naphthyl acetate) (3.1.1.-)	<i>Es-1</i>	LiOH
	<i>Es-4</i>	LiOH
	<i>Es-5</i>	LiOH
Glucose-6-phosphate dehydrogenase (1.1.1.49)	<i>G6pdh</i>	TC 8.0
Glucose-6-phosphate isomerase (5.3.1.9)	<i>Gpi</i>	TC 8.0
Isocitrate dehydrogenase (1.1.1.42)	<i>Idh-1</i>	TC 6.0
	<i>Idh-2</i>	TC 6.0
Leucine aminopeptidase (3.4.11.-)	<i>Lap-1</i>	LiOH
	<i>Lap-2</i>	LiOH
Malate dehydrogenase (NAD) (1.1.1.37)	<i>Mdh-1</i>	LiOH
	<i>Mdh-2</i>	LiOH
Malate dehydrogenase (NADP+) (1.1.1.40)	<i>Mdhp-1</i>	LiOH
	<i>Mdhp-2</i>	LiOH
Peptidase (L-leucyl-L-alanine) (3.4.-)	<i>Pep-A-1</i>	TC 6.0
	<i>Pep-A-2</i>	TC 6.0
Peptidase (L-leucylglycylglycine) (3.4.-)	<i>Pep-B-2</i>	LiOH
	<i>Pep-B-3</i>	LiOH
Phosphoglucosmutase (5.4.2.2)	<i>Pgm-1</i>	TC 8.0
	<i>Pgm-2</i>	TC 8.0
6-Phosphogluconate dehydrogenase (1.1.1.44)	<i>Pgdh</i>	TC 6.0
Xanthine oxidase (1.1.1.204)	<i>Xdh</i>	TC 6.0

*TC 6.0: 0.188 M Tris, 0.065 M citrate, adjusted to pH 6.0; diluted 1:9 for gels and 1:5 for electrodes (14 hr, 80 v). LiOH. solution A: 0.03 M LiOH, 0.19 M borate, pH 8.1; solution B: 0.008 M citrate, 0.05 M Tris, pH 8.4; 1A:9B for gels, A for electrode. TC 8.0. electrode: 0.678 M Tris, 0.157 M citrate, adjusted to pH 8.0; gel: 22.89 mM Tris, 5.22 mM citrate, pH 8.0 (14 hr, 100 v).

sample was $\bar{P} = 4.59\%$ (range: 0.0-12.5%). Mean individual heterozygosity ($\bar{H}$) was also very low: $\bar{H} = 0.011$ (range: 0.000-0.025) with one outlier at Mekong River-5 (sample 15) where $H = 0.058$. As 24 loci were surveyed in every sample and as mean sample size per locus was 21-28, our results are adequate to conclude that the Thai *Corbicula* have very little genetic variability.

Minor genetic variation was detected at 28 localities (70%) around Thailand; the remaining 12 were isogenic. Sixteen samples had 1 polymorphic locus, 8 had 2 polymorphic loci, and 4 had 3 polymorphic loci. This variation was detected in 5 loci, four of which were di-allelic and one was tri-allelic. In no case, however, were more than two alleles detected at a locus in a single population. There was no discernible geographic pattern to this variation although levels of P were slightly higher in the northeast ($\bar{P} = 0.065$) than elsewhere ($P = 0.03 - 0.046$).

Where a locus was polymorphic at a particularly locality, the observed genotype frequencies were compared with the frequencies expected under panmixia. Using the chi-

square test, the observations were in agreement with Hardy-Weinberg expectations in 40 out of 43 tests ($P > 0.05$). In the remaining three cases, two (both involving *Lap-2*) were also in agreement with Hardy-Weinberg expectation when the Fisher exact test was used. Only one case (involving *Es-5* in sample 15) failed both tests: we detected 6 AA, 24 AB and no BB individuals. This accounts for the elevated heterozygosity noted at this locality.

In the Thai *Corbicula*, we found the simple morphological dichotomy between white and purple internal shell color to be generally inapplicable (Kijviriya, 1990); like Morton (1987) in Hong Kong, we recognized more intermediate color types. However, in eight parts of Thailand we found populations with approximately bimodal distributions of color morphs (samples: 9, 11, 15, 18-19 and 35-37). When these morph-sorted subsamples were compared they were found to be indistinguishable at the 24 allozymic loci. The genes we examined included three of the six loci found to show fixed differences between the contrasting morphotypes studied in Texas by Hillis and Patton (1982).

Table 2. Allele frequencies for variable loci* in 40 samples of Thai *Corbicula*, with summary statistics of genetic variability at 24 loci**.

Sample	<i>Es-5</i> ^a	<i>Lap-2</i> ^a	<i>Mdh-1</i> ^a	<i>Pep-1</i> ^a	<i>Pgdh</i> ^c	$\bar{N}$	$\bar{A}$	P	$\bar{H}$
1	1.00	0.95	1.00	0.80	0.93	28	1.1	0.13	0.02
2	1.00	1.00	1.00	1.00	1.00	25	1.0	0.00	0.00
3	1.00	1.00	0.83	1.00	1.00	26	1.0	0.04	0.01
6	1.00	0.88	1.00	0.95	1.00	24	1.1	0.08	0.01
7	1.00	0.93	1.00	1.00	1.00	26	1.0	0.04	0.003
9	0.90	0.93	0.92	1.00	1.00	25	1.1	0.13	0.02
10	1.00	1.00	0.88	1.00	1.00	25	1.0	0.04	0.01
11	1.00	1.00	0.97	1.00	0.78	26	1.1	0.08	0.02
12	1.00	1.00	1.00	0.87	1.00	26	1.0	0.04	0.01
13	1.00	1.00	1.00	0.85	1.00	27	1.0	0.04	0.01
14	1.00	1.00	1.00	0.87	1.00	26	1.0	0.04	0.01
15	0.60	0.85	1.00	0.85	1.00	25	1.1	0.13	0.06
16	1.00	0.87	0.93	0.95	1.00	27	1.1	0.13	0.02
18	0.23	1.00	1.00	0.93	1.00	26	1.1	0.08	0.03
19	0.10	0.95	1.00	1.00	1.00	26	1.1	0.08	0.01
20	0.00	0.85	1.00	0.80	1.00	26	1.1	0.08	0.02
21	0.00	1.00	1.00	0.85	1.00	26	1.0	0.04	0.01
22	0.20	1.00	1.00	0.90	1.00	25	1.1	0.08	0.03
24	0.97	1.00	0.85	1.00	1.00	24	1.1	0.08	0.02
25	0.80	1.00	1.00	1.00	1.00	25	1.0	0.04	0.02
26	0.97	1.00	1.00	1.00	1.00	25	1.0	0.04	0.003
28	1.00	0.83	1.00	1.00	1.00	25	1.0	0.04	0.01
31	1.00	1.00	0.78	1.00	1.00	25	1.0	0.04	0.02
32	1.00	1.00	1.00	0.87	1.00	24	1.0	0.04	0.01
33	1.00	0.90	1.00	0.92	1.00	25	1.1	0.08	0.02
34	1.00	0.92	1.00	1.00	1.00	26	1.0	0.04	0.01
35	1.00	0.82	1.00	1.00	1.00	26	1.0	0.04	0.02
36	1.00	0.87	1.00	1.00	1.00	24	1.0	0.04	0.01
39	1.00	0.88	1.00	1.00	1.00	23	1.0	0.04	0.01

*Each variable locus has two alleles except for *Pgdh* where *c/a* and *c/b* occur in samples 1 and 11, respectively. All other loci (see Table 1) were monomorphic.

**Samples described in the Appendix. The following 11 samples are not shown but were identical to sample 2: 4, 5, 8, 17, 23, 27, 29, 30, 37, 38, 40 (i.e. $N = 22-27$, $A = 1.0$, $P = 0$, $H = 0$), where N = mean sample size per locus, A = mean no. alleles per locus, P = proportion of loci polymorphic, H = mean individual heterozygosity.

We tested the relationship between genic variability (P) and local abundance by simple regression analysis. As expected, we found a significant negative correlation between abundance and sample area ($r^2 = 0.4$). There was, however, no significant relationship between sample area or abundance and P ($r^2 = 0.13$ and 0.09 , respectively). Population variability was not simply a function of population size.

We looked for ecological factors that could be associated with locally elevated levels of P . The four most variable samples (samples 1, 9, 15 and 16) showed no consistent pattern with respect to water depth, water temperature, pH or habitat type. We are unable to distinguish such sample sites ecologically from sites with isogenic clams.

Genetic relationships between the 40 samples were studied by calculating various measures of genetic distance and clustering these values using various tree building algorithms. A phenogram (not shown) based on Nei's genetic distance (D) did not discriminate between almost 90% of the samples; one based on Rogers' genetic similarity resolved the trivial differences between some samples (Fig. 2). Both algorithms show that all the *Corbicula* studied from throughout Thailand are virtually identical to one another at the 24 allozyme loci examined.

DISCUSSION

The interpretations concerning allelic variation were made in a very conservative fashion. Genetically interpretable variation was seen in 5 loci: *Es-5*, *Lap-2*, *Mdh-1*, *Pep-A-1* and *Pgdh*. In the majority of cases (39 out of 44), where a locus was polymorphic at a particular locality, only one of the two homozygote genotypes was observed. Both homozygote genotypes were, however, seen in at least one sample for 4 of the 5 polymorphic loci (*Es-5* being the exception). Thus, our genetic interpretations are supported by the observation of all possible genotypes segregating in Thai *Corbicula* in the majority of the variable allozymes.

Another laboratory using different techniques could find variation where we have found none as single-gel electrophoretic surveys are known to fail to detect about 20% of true sample variability (Ayala, 1982; Selander and Whittam, 1983). For this and other reasons, the comparison of electrophoretic results between different laboratories is not recommended (Nei, 1987). However, some comments on the results of Smith *et al.* (1979), the only other published survey of variation in Asian *Corbicula*, are appropriate. Their study involved eight unique loci and ten that were shared with our survey. They too found variation in *Mdh-1* and *Pgdh* in Asian *Corbicula*. In contrast, where they found *Gpi-1* was variable in samples from Japan, Hong Kong and the Philippines, we detected no variation in Thailand. The other three polymorphic loci that we detected in Thailand were not studied by Smith *et al.* (1979).

The number of individual clams studied per sample and the number of loci investigated per clam are adequate for the estimation of levels of genic variability. The approximate average values of $\bar{A} < 1.1$, $\bar{P} = 0.05$ and $\bar{H} = 0.01$ are all very low relative to those estimated for many other

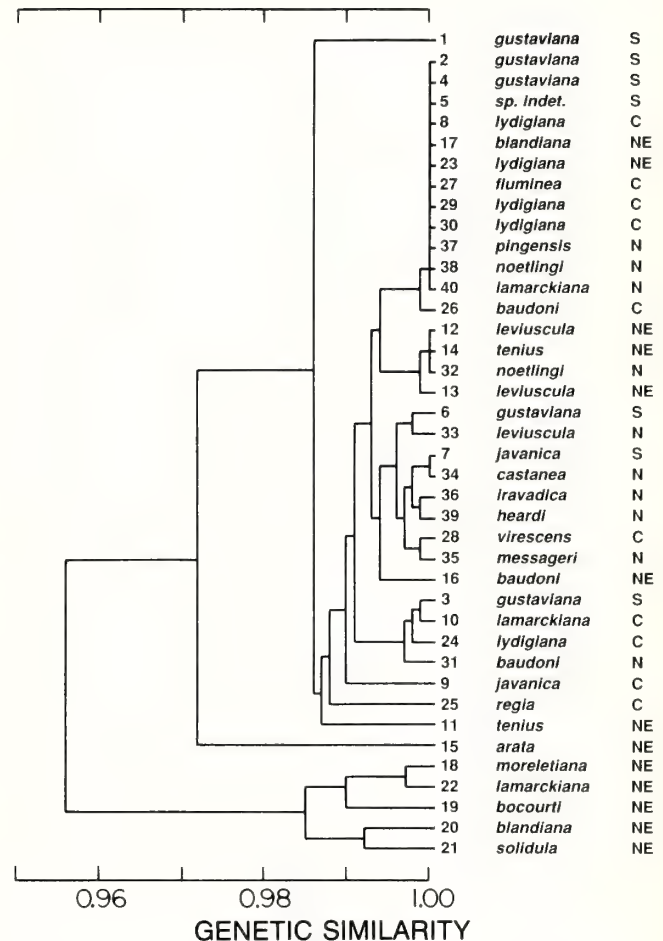


Fig. 2. Dendrogram showing relationships among 40 samples of Thai *Corbicula* generated by UPGMA clustering of Rogers' (1972) genetic similarity (S) estimates based on 24 loci. The cophenetic correlation is 0.971.

species of invertebrates. For example, Nevo *et al.* (1984) reported that for 361-371 species of invertebrates $\bar{P} = 0.38$ and $\bar{H} = 0.10$. Our estimates for the Thai *Corbicula* are also lower than those obtained by Smith *et al.* (1979) who found $P = 0.17$ - 0.76 in the Philippines, Hong Kong and Japan. The average Thai values are thus closer to those estimated for North American populations where $\bar{P} = 0.04$ and $\bar{H} = 0.002$ (data summarized by Britton and Morton, 1986). Such mean values can, however, be misleading: the most variable sample in Thailand (sample 15) had $P = 0.125$ and $\bar{H} = 0.06$ and is thus much more variable than most of the North American populations.

Corbicula fluminea has, at various times, been regarded as dioecious, as monoecious, as a consecutive protandic hermaphrodite, and as a simultaneous hermaphrodite (Kraemer, 1979; Britton and Morton, 1979, 1986). The low level of population variability and very low individual heterozygosity suggest that most of the *Corbicula* in Thailand are facultative self-fertilizers. The reduced variability observed is similar to that found in other self-fertilizing molluscs (Selander and Kaufman, 1973; Selander and Hudson, 1976; Hornbach *et al.*, 1980;

McCracken and Selander, 1980; Stoddard, 1983; Nevo *et al.*, 1984). Morton (1983) recognized that different populations of *C. fluminea* can have different sexual strategies; he noted different degrees of self-fertilization in lotic and lentic habitats.

The small amount of genic diversity detected in 70% of the samples must be maintained by either inbreeding among a few strains with limited outcrossing or by the self-fertilization of a large number of heterozygotes. We favor the former explanation as we failed to find the alternate homozygotes in 88% of the situations where we detected heterozygotes (each situation involved a specific polymorphic locus in a specific sample). Occasional random outcrossing would also account for observations that genotype frequencies conformed to Hardy-Weinberg expectations in 98% of the cases and the concomitant lack of a consistent pattern of heterozygote deficiencies expected for obligate self-fertilizing species.

Linkage disequilibrium, which is common in self-fertilizing plants, was not detected in the variable samples of *Corbicula*. There was no significant association between alternate alleles at two or more polymorphic loci in any population. Thus, the variable populations in Thailand are not simply composed of two or more monogenic clones with limited interbreeding. Instead the pattern we discovered suggests amphimixis is occurring today in some populations that have a recent history of automixis. The proportion of progeny produced by self-fertilization (S) can be estimated from the proportion of heterozygous individuals in each subpopulation (Hamrick, 1983; Hillis, 1989). Using *Pgdh* genotype frequencies we estimated the frequency of self-fertilization in sample 10 to be 13%; using *Pep-A-1* we estimated the rates to be 29% in sample 1, 29% in sample 20, and 23% in sample 14; using *Lap-1* our estimates were 68% in sample 7 and 57% in sample 16. These results indicate that multiple reproductive modes may be used differentially in different localities.

The very low levels of genic variation and small sample sizes make it difficult to study population structure using Wright's (1978) fixation indices or F -statistics. The over-all loci values of $F_{is} = -0.13$ indicates insignificant inbreeding within each sample. However, when the data are examined on a finer scale, eight significant individual locus F_{is} values were obtained in eight different samples; i.e. $F_{is} > -0.20$ for *Es-5* in four samples, and for *Lap-2* and *Mdh-1*, in two different samples each. In contrast, the larger $F_{st} = 0.46$ indicates significant variation among samples. This value is similar to the average fixation index for self-fertilizing plants ($F_{st} = 0.44$; Hamrick, 1983). The reduction in individual heterozygosity in the Thai *Corbicula* as a whole (due to both substructuring and inbreeding) is also quite substantial ($F_{it} = 0.39$).

The phenetic tree based on Rogers' genetic similarity (Fig. 2) shows how all 40 samples of Thai *Corbicula* are almost identical at the loci studied. In terms of the more widely reported Nei's genetic distances, 88% of the samples (35/40) cluster at averaged genetic distance values of $\bar{D} < 0.01$; such values are smaller than their associated standard errors (Nei *et al.*, 1986). Most of the Thai *Corbicula* are thus genetically identical or indistinguishable at a reasonably large sample of allozyme loci. Only five samples from northeast Thailand

stand slightly apart from the others: samples 20-22 from the Mun River drainage and samples 18-19 from the Mekong River at Nakhon Phanom. Their genetic differentiation rests primarily on their having unusually high frequencies of *Est-5b* and to a lesser extent on their frequencies of *Pep-A-1b*. Neither of these alleles are unique to these localities or even to north-east Thailand, however, so these samples are differentiated from the others at a barely significant level ($D = 0.03-0.04$). Not only were these regionally commoner alleles found elsewhere in Thailand, but we failed to detect them at two other Mekong River sites (samples 14-15) between Nakhon Phanom and the point 300 km downstream where the Mun River enters the Mekong.

The overall level of genetic differentiation seen among the 40 samples of *Corbicula* is well within that expected for conspecific populations of sexually reproducing species. Thorpe's (1983) survey of 1111 published conspecific comparisons showed that 98% of the genetic distance estimates were $D \leq 0.10$. Self-fertilizing species have received less attention, but generally show more geographic differentiation than outcrossing species, i.e. the observed values for Thai *Corbicula* are much lower than expected for a group of self-fertilizing congeneric species. The very high genetic similarities estimated here provide no support for the recognition of more than one species of *Corbicula* in our samples.

We hasten to add that even within a clade there is no simple relationship between genetic distance and taxonomic level. Some most closely related species are known that are very similar at their allozyme loci (Davis *et al.*, 1981; Gould and Woodruff, 1987). Nevertheless, the preponderance of the evidence on genetic differentiation associated with species of vertebrates (Avice and Aquadro, 1982), invertebrates generally (Nei, 1987), and molluscs in particular (Woodruff *et al.*, 1988), supports the conclusion that our samples are conspecific. Furthermore, the degree of differentiation seen in the Thai *Corbicula* is less than that reported in other species of freshwater bivalve (Davis, 1983; Davis *et al.*, 1981; Kat, 1983; Kat and Davis, 1984). It is significantly less than that reported between clones of other automictic molluscs ($D = 0.06-0.12$) by McCracken and Selander (1980). The lack of genic differentiation in an area the size of Thailand was a little surprising. We failed to detect any evidence for even incipient speciation in clams from different river systems. Had we known, *a priori*, that the Thai *Corbicula* were primarily self-fertilizing we would have predicted even more differentiation between clones; as was found in North American slugs (McCracken and Selander, 1980) and Australian *Thaïra* Stoddard, 1983, 1985).

In the absence of comparable allozyme studies of *Corbicula* from elsewhere in Southeast Asia, we are unable to discuss the evolution or phylogenetic relationships of the Thai clams. Their close similarity probably reflects recency of divergence. Time for the accumulation of genetic variation has been limited and self-fertilization has worked constantly to eliminate heterozygotes and rare alleles. The degree of differentiation observed among the Thai *Corbicula* could have evolved in less than 200,000 years if Nei's (1987) moderate calibration of the protein-electrophoretic clock (with a D value

of 1.0 equivalent to about 5 million years) is applicable.

The allozymic similarity of the 40 samples of Thai *Corbicula*, together with their lack of taxonomically significant anatomical or conchological variation (Kijviriyā, 1990), indicates that all are referable to a single species. Two arguments can be made that this species is *C. fluminea*. First, Morton (1986) and Britton and Morton (1986) have argued that most, if not all, of the Asian freshwater *Corbicula* are synonyms of *C. fluminea*. Second, Brandt (1974) identified *C. fluminea* as occurring at several localities in Thailand and we have studied variation in clams from one of these localities (sample 27) and found that morphologically they are typical of *C. fluminea*. Furthermore, as we have discovered that these clams are genetically indistinguishable from all other *Corbicula* studied in Thailand, we propose the following taxa be synonymized with *C. fluminea* (Müller, 1774):

Corbicula arata (Sowerby, 1877) (Brandt, 1974:313; pl. 27, fig. 73)

C. baudoni Morelet, 1866 (Brandt, 1974:323; pl. 29, fig. 102)

C. blandiana Prime, 1864 (Brandt, 1974:313; pl. 27, fig. 72)

C. bocourti (Morelet, 1865) (Brandt, 1974:314; pl. 27, fig. 80)

C. castanea (Morelet, 1865) (Brandt, 1974:317; pl. 27, fig. 79)

C. gustaviana Martens, 1900 (Brandt, 1974:320; pl. 28, fig. 87)

C. heardi Brandt, 1974:328; pl. 29, fig. 104

C. iravadica Hanley and Theobald, 1876 (Brandt, 1974:323; pl. 28, fig. 91)

C. javanica (Mousson, 1849) (Brandt, 1974:315; pl. 27, fig. 82)

C. lamarckiana Prime, 1864 (Brandt, 1974:316; pl. 27, fig. 76-77)

C. leviuscula Prime, 1864 (Brandt, 1974:326; pl. 28, fig. 95)

C. lydigiana Prime, 1861 (Brandt, 1974:316; pl. 27, fig. 74-75)

C. messengeri Bavay and Dautzenberg, 1901 (Brandt, 1974:327; pl. 29, fig. 100)

C. moreletiana Prime, 1867 (Brandt, 1974:321; pl. 28, fig. 89-90)

C. noetlingi Martens, 1899 (Brandt, 1974:319; pl. 28, fig. 88)

C. pingensis Brandt, 1974:324; pl. 28, fig. 93

C. regia Clessin, 1879 (Brandt, 1974:320; pl. 28, fig. 86)

C. solidula Prime, 1861 (Brandt, 1974:326; pl. 28, fig. 96)

C. tenuis Clessin, 1887 (Brandt, 1974:318; pl. 28, fig. 85)

C. virescens Brandt, 1974:324; pl. 29, fig. 101

As Brandt (1974) has provided fully referenced synonyms, notes on conchology, types, localities and distribution, and excellent photographs of representative shells, on the pages and plates cited above, such data are not repeated here. Kijviriyā (1990) also provides color photographs of representative shells (see Appendix for specific figure numbers).

These taxonomic conclusions are based on the Thai material examined. We cannot assess the validity of any of these 21 taxa from elsewhere in Asia, e.g. *Corbicula gustaviana* from Sumatra or *C. javanica* from Java. In addition, the following seven nominal Thai species were not collected (*C. cyreniformis* Prime, 1860; *C. gubernatoria* Prime, 1869; *C. occidentiformis* Brandt, 1974; *C. pisidiformis* Prime, 1866; *C. siamensis* Prashad, 1929; *C. vokesi* Brandt, 1974) or were found in inadequate numbers for electrophoretic analysis (*C. erosa* Prime, 1861). We therefore have no opinion as to the validity of these taxa but suspect that they too are junior synonyms of *C. fluminea*.

This, then, is our principal result: the freshwater clams of the genus *Corbicula*, collected from sites up to 1500 km apart in Thailand, show no significant geographic variation at 24 genetic loci and are referable to the widespread Asian species *C. fluminea*. This result would be confirmed if it were shown that the Thai *Corbicula* are closely related to better known *C. fluminea* from Hong Kong and North America. We predict that the Chinese clams will be very similar genetically to those in Thailand ($D < 0.10$). Those of the Philippine islands could show greater differentiation associated with founder effects and perhaps reduced variability as in the case of the introduced North American clams. The reported higher levels of variation in Japan ($P = 0.76$, $\bar{H} = 0.23$; Smith *et al.*, 1979) are paradoxical and require confirmation. Perhaps the radically different level of variation in this peripheral population indicates that the Japanese clams have adopted a different mating strategy to those used elsewhere. Alternatively, the island could have been colonized repeatedly by clams of diverse geographical origin.

In parts of the United States, two morphotypes of *Corbicula fluminea* have been identified based on differences in color of the nacre: white or dark purple. The typical form has colored shells and the dark form has purple shells. Hillis and Patton (1982) found that in Texas the two morphs had fixed differences at six of 26 allozyme loci. McLeod (1986) reported a similar finding. Although Hillis and Patton (1982) and others have used these genetic differences as evidence for the presence of two species in North America, the analyses of Britton and Morton (1986) favor a one-species model. Britton and Morton argued that other conchological, ecological and behavioral traits are associated with this dimorphism in both North America and Hong Kong. These suites of associated characters are apparently local adaptive responses to environmental variables and the contrasting morphotypes are thus better regarded as ecophenotypes. According to Britton and Morton (1986), allozymic variation has now been described in 22 North American populations of the white morphotype; $\bar{P} = 0.04$ (range = 0-19%) and $\bar{H} = 0$ in 17 populations and $\bar{H} = 0.0013-0.0049$ in 5 populations. The 3 samples of the purple morphotype that have been described were all monomorphic ($P = 0$, $\bar{H} = 0$). No other genetic studies have been published, although work in progress on rRNA variation will reopen the question of whether more than one species is present in North America (D. Hillis, pers. comm.).

A paradox to emerge from our study concerns *Corbicula fluminea*'s great ecological success despite its innate lack of genetic variability. We have found that one of the commonest bivalves is as genetically depauperate in parts of its native range as it is in areas it has successfully colonized in the last 50 years. Although some workers have maintained that genetic variability is positively related to ecological and evolutionary adaptability, it is clear that *C. fluminea*'s success is based on a narrow genetic base. McCracken and Selander (1980) found a similar pattern in some European slugs introduced into North America. Homozygous self-fertilizing species of slugs occupied a wider range of habitats and had superior colonizing abilities than some more heterozygous outcross-

ing species. *Corbicula* appears to have such a less variable general purpose genome. Clearly, the larger issues presented by *Corbicula* cannot be resolved from within Thailand; a broader geographic survey of genic variability and breeding systems in this widespread species is now required. Only when this is done will we begin to understand the relationship between genetics, the environment, and *C. fluminea*'s marked conchological, ecological and behavioral variation.

ACKNOWLEDGMENTS

We thank Captain Vinyoo Kijviriyaa, RTN, Dr. Usa Klinhom, Miss Duangduen Ratanaponglakha, Mr. Thongleum Saesonthi and Mr. Somyos Hatsanate for their assistance with collecting clams. Vimon Sri Manasarn and M. Patricia Carpenter provided excellent technical assistance in the laboratory in Bangkok and San Diego, respectively. The senior author was supported by Ramkhamhaeng University and the National Research Council of Thailand and acknowledges the advice of Drs. Maleeya Kruatrachue and Rojana S. Keawjam. Work in San Diego was supported by the Academic Senate of the University of California and, indirectly, by grants from the U.S. National Science Foundation, to DSW. We thank David M. Hillis and an anonymous reviewer for their comments on the manuscript.

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Date of manuscript acceptance: 4 February 1991

APPENDIX

The 40 samples are listed by number (1-40, in boldface type); locality; (relative abundance); and species name according to criteria of Brandt (1974). These sample numbers are placed by locality on the map of Thailand (Fig. 1). Clam abundance was scored on a scale from 1 (rare) to 5 (superabundant), where absolute densities per square meter are approximately 10, 100, 500, 1000, and > 1000. In brackets following each species name, we give the voucher specimen's lot number together with a reference to the color photograph (plate and figure number) of representative shells in Kijiviriya (1990). Shells were given Mahidol University, Faculty of Science (MUFs) code numbers and deposited in the Museum of the Center for Applied Malacology and Entomology, Mahidol University.

1. Lake at Lampam, Phattalung province, (5), *Corbicula gustaviana* (MUFs-TH00-155, pl. 2, fig. 8B).
2. Ta Pi River-1, Chawang district, Nakhon Si Thammarat province, (2), *C. gustaviana* (MUFs-TH00-157, pl. 2, fig. 8D).
3. Min canal, Chawang district, Nakhon Si Thammarat province, (2), *C. gustaviana* (MUFs-TH00-156, pl. 2, fig. 8C).
4. Ta Pi River-2, Lampoo island, Muang district, Surat Thani province (3), *C. gustaviana* (MUFs-TH00-158, pl. 2, fig. 8E).
5. Tachin River, Muang district, Trang province, (4), *C. sp. indet.* (MUFs-TH00-191, pl. 4, fig. 23).
6. Ngae canal, Khlong Ngae Village, Sadao district, Songkhla province, (4), *C. gustaviana*. (MUFs-TH00-154, pl. 2, fig. 8A).
7. Saiburi River, Saiburi district, Pattani province, (5), *C. javanica* (MUFs-TH00-163, pl. 2, fig. 11A/B).
8. Maeklong River, Muang district, Ratchaburi province, (3), *C. lydigiana* (MUFs-TH00-168, pl. 2, fig. 12C).
9. Phetchaburi River, Muang district, Phetchaburi province, (2), *C. javanica* (MUFs-TH00-164, pl. 2, fig. 11C).
10. Phetchaburi irrigation canal, Muang district, Phetchaburi province, (2), *C. lamarckiana* (MUFs-TH00-172, pl. 3, fig. 13C).
11. Mekong River-1, Khemmarat district, Ubon Ratchathani province, (2), *C. tenuis* (MUFs-TH00-189, pl. 4, fig. 21C).
12. Mekong River-2, Huai Klor, Nong Khai province, (3), *C. leviuscula* (MUFs-TH00-176, pl. 3, fig. 14C).
13. Mekong River-3, Phaeng village, Nakhon Phanom province, (3), *C. leviuscula* (MUFs-TH00-177, pl. 3, fig. 14D).
14. Mekong River-4, Muang district, Mukdahan province, (4), *C. tenuis* (MUFs-TH00-188, pl. 4, fig. 21B).
15. Mekong River-5, Pakchom district, Nong Khai province, (3), *C. arata* (MUFs-TH00-142, pl. 1, fig. 1).
16. Sui River, near Nong Khai town, Nong Khai province, (5), *C. baudoni* (MUFs-TH00-144, pl. 1, fig. 2B).
17. Loei River, Muang district, Loei province, (1), *C. blandiana* (MUFs-TH00-148, pl. 1, fig. 3A).
18. Pho canal-1, Huai Pho, Nakhon Phanom province, (5), *C. moreletiana* (MUFs-TH00-179, pl. 3, fig. 16A/B).
19. Pho canal-2, Huai Pho, Nakhon Phanom province, (5), *C. bocourti* (MUFs-TH00-150, pl. 1, fig. 4).
20. Mun River, Phibun Mangsahan district, Ubon Ratchathani province, (4), *C. blandiana* (MUFs-TH00-149, pl. 1, fig. 3B).
21. Pan River, Yang Talat district, Kalasin province, (3), *C. solidula* (MUFs-TH00-186, pl. 4, fig. 2).
22. Chi River, Muang district, Maharakham province, (3), *C. lamarckiana* (MUFs-TH00-173, pl. 3, fig. 13D).
23. Ubonrat reservoir, Khon Kaen province, (3), *C. lydigiana* (MUFs-TH00-167, pl. 2, fig. 12B).
24. Panthong canal, Panthong village, Chonburi province, (4), *C. lydigiana* (MUFs-TH00-170, pl. 2, fig. 12E).
25. Pra Sae Bon River, Glaeng district, Rayong province, (3), *C. regia* (MUFs-TH00-185, pl. 4, fig. 19A/B).
26. Chao Phraya River-1, Pamoke district, Angthong province, (3), *C. baudoni* (MUFs-TH00-146, pl. 1, fig. 2D).
27. Chao Phraya River-2, Krung Thon bridge, Sanghae area, Bangkok, (3), *C. fluminea* (MUFs-TH00-153, pl. 1, fig. 7A/B).
28. Chao Phraya River-3, Muang district, Nakhon Sawan province, (4), *C. virescens* (MUFs-TH00-190, pl. 4, fig. 22).
29. Chao Phraya River-4, Kwae Noi River, Sena district, Ayutthaya province, (3), *C. lydigiana* (MUFs-TH00-169, pl. 3, fig. 12D).
30. Irrigation canal, Lopburi town, Lopburi province, (3), *C. lydigiana* (MUFs-TH00-166, pl. 2, fig. 12A).
31. Ping River-1, Wuttikul bridge, Tak province, (3), *C. baudoni* (MUFs-TH00-147, pl. 1, fig. 2E).
32. Ping River-2, Naowaraj bridge, Chiang Mai province, (3), *C. noetlingi* (MUFs-TH00-180, pl. 4, fig. 17D).
33. Ping River-3, Naowaraj bridge, Chiang Mai province, (3), *C. leviuscula* (MUFs-TH00-175, pl. 3, fig. 14B).
34. Yom River, Muang district, Prae province, (4), *C. castanea* (MUFs-TH00-151, pl. 1, fig. 5).
35. Kuang River, Muang district, Lamphun province, (3), *C. messengeri* (MUFs-TH00-178, pl. 3, fig. 15).
36. Moei River-1, Ban Takham, Tak province, (2), *C. iravadica* (MUFs-TH00-162, pl. 2, fig. 10B).
37. Moei River-2, Mae Sot district, Tak province, (1), *C. pingensis* (MUFs-TH00-183, pl. 4, fig. 18A).
38. Fang River, Fang district, Chiang Mai province, (3), *C. noetlingi* (MUFs-TH00-181, pl. 4, fig. 17C).
39. Mae Lao River, Muang district, Chiang Rai province, (1), *C. heardi* (MUFs-TH00-160, pl. 2, fig. 9B).
40. Kaek River, Wang Thong district, Phitsanuloke province, (2), *C. lamarckiana* (MUFs-TH00-171, pl. 3, fig. 13A/B).

SHELL AND HINGE DEVELOPMENT OF YOUNG *CORBICULA FLUMINEA* (MÜLLER) (BIVALVIA: CORBICULOIDEA)

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ABSTRACT

Small specimens of *Corbicula fluminea* (Müller) were collected as juveniles newly released from adults, and larger specimens were extracted from sediment taken from natural beds of adults. Lengths of the prodissoconch I averaged $196.9 \pm 6.7 \mu\text{m}$ ($\bar{x} \pm \text{SD}$; range = 187-213 μm ; $n = 17$); the average length for the prodissoconch II was $233.3 \pm 5.0 \mu\text{m}$ (range 228-244 μm , $n = 10$). The dissoconch had strong commarginal sculpturing, with less prominent radial striae also present. A ligament pit occurred in the posterior region of the shell hinge. Three cardinal and two lateral teeth developed, and shell tubules were noted in the early dissoconch.

Over at least the last 65 years (McMahon, 1982; Counts, 1986), an introduced bivalve generally recognized as *Corbicula fluminea* (Müller) (Britton and Morton, 1986; but see Hillis and Patton, 1982; McLeod, 1986) has spread throughout the lower U.S. mainland, including sites in Maryland's watershed surrounding Chesapeake Bay (Counts, 1981; Kennedy and Van Heukelem, 1985). Adults brood eggs in demibranchs and release juveniles whose shell and hinge development we report on here.

MATERIALS AND METHODS

Adult *Corbicula fluminea* were collected in September in Nassawango Creek, Maryland, a short tributary of the Pocomoke River (approx. 38° 10' N; 75° 27' W), and were held in the laboratory in glass bowls of fresh water. Juvenile clams that they released were preserved in 95% ethanol. In addition, sediment taken from the clam bed later in November was sieved (500 μm) to retain sand and small clams that were collected with a pipette under a dissecting microscope. These

small clams, and larger juveniles collected from Whites Ferry in the Potomac River, Maryland (approx. 39° 09' N; 77° 31' W) in June, were also preserved in 95% ethanol.

Preserved clams were prepared for scanning electron microscopy (Fuller *et al.*, 1989) by placement in 5.25% sodium hypochlorite for about 10 min to remove soft tissues. The resulting separated shells were rinsed in distilled water. Specimens less than or about 250 μm long were mounted on silver tape, with double coated tape used to prevent charring in larger shells. Specimens were coated with about 600 Å of gold-palladium and examined under an ETEC Autoscan scanning electron microscope. Orthogonal accuracy (i.e. equal magnification in horizontal and vertical directions) and accuracy in measurements were obtained by careful initial calibration of the horizontal and vertical magnifications, and by orienting shells such that anterior and posterior as well as dorsal and ventral margins were at equal working distances from the electron optical system (Fuller *et al.*, 1989). Shell dimensions were determined by comparison with standard grids photographed at the same magnification as each shell. Length measurements were made from the extreme anterior to posterior margins of each prodissoconch I, prodissoconch II, or dissoconch.

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RESULTS AND DISCUSSION

Shortly after recently collected adults were placed in glass bowls of fresh water, groups of discharged young could be seen on the bottom of the bowls near the parents. As reported earlier (Kennedy and Van Heukelem, 1985), newly released clams measured 210 - 250 μm long ($\bar{x}$ = 236 μm , n = 12). They had a relatively straight hinge (Fig. 1), and exhibited the D-shape common to many bivalve larvae. The prodissoconch I and much if not all of the commarginal prodissoconch II (Fig. 1) apparently had formed while the young were being brooded. The dissoconch, produced after release, exhibited a strong commarginal sculpture, with less prominent radial striae also present (Fig. 2). This external appearance of the juvenile shell is of interest in that the shells of larger individuals do not exhibit such radial striae, whereas the commarginal sculpture remains prominent. The transitions in external appearance from a shell surface with no growth lines (prodissoconch I) to surfaces with increasingly distinct growth lines (prodissoconch II, dissoconch) are shown in figure 3. Range of length for prodissoconch I was 187 - 213 μm ($\bar{x} \pm \text{SD}$ = 196.9 $\pm$ 6.7 μm , n = 17) and for prodissoconch II was 228 - 244 μm ($\bar{x}$ = 233.3 $\pm$ 5.0 μm , n = 10).

Shells gradually changed from D-shaped to oval as the umbones developed (400 - 600 μm , Fig. 4), with the anterior margin becoming more prominent than the posterior margin over time (at least by about 600 μm in length, Fig. 4). A change in shell shape occurs with growth: larger or adult clams possess a rounder anterior margin and more pointed posterior margin. The adductor muscle scars on the internal surface of most shells in figure 4 reflect the difference in shape between the more rounded posterior and more elongate anterior adductor muscles (see also Britton and Morton, 1982).

Internally, no denticles appeared in the provinculum (hinge) of newly released pediveligers (Fig. 5). Thereafter, a

posterior ligament pit was first apparent at 257 μm , becoming prominent at 411 μm (Fig. 5). The appearance of the ligament pit after the dissoconch began to develop supports Lutz and Hidu's (1979) hypothesis that development of this pit is one of the earliest changes to occur in shell morphology during metamorphosis in bivalves. Two lateral teeth and three cardinal teeth (anterior, central, posterior) were fully formed in each valve at lengths of 2 mm (Figs. 4, 5, 6).

Small numbers of shell tubules (Tan Tiu and Prezant, 1989) can be seen in the internal shell surfaces of all but the smallest shells (229, 257 μm) in figure 4 (see also Figs. 5, 6). This complements Tan Tiu and Prezant's (1989) report on tubules in shells of 7 to 38 mm long clams. Such tubules could develop only after metamorphosis in *Corbicula fluminea*,



Fig. 2. Scanning electron micrograph of the exterior shell surface of the right valve of a 685 μm specimen of *Corbicula fluminea*, showing strong commarginal and weaker radial sculpture of the dissoconch.

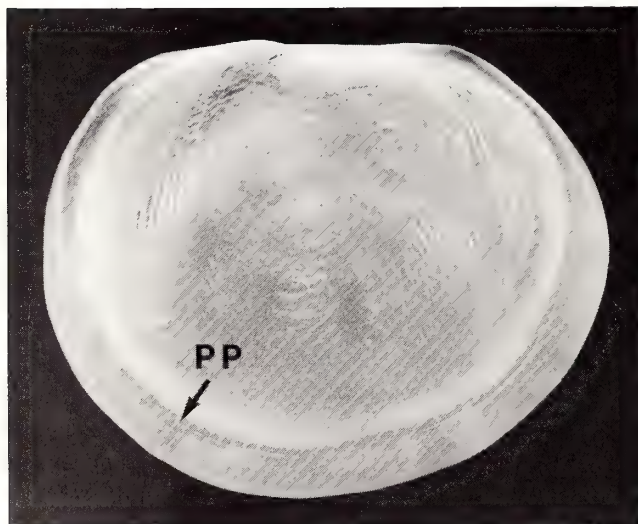


Fig. 1. Scanning electron micrograph of the exterior shell surface of the left valve of a 233 μm specimen of *Corbicula fluminea*. PP marks the prodissoconch I - prodissoconch II boundary.

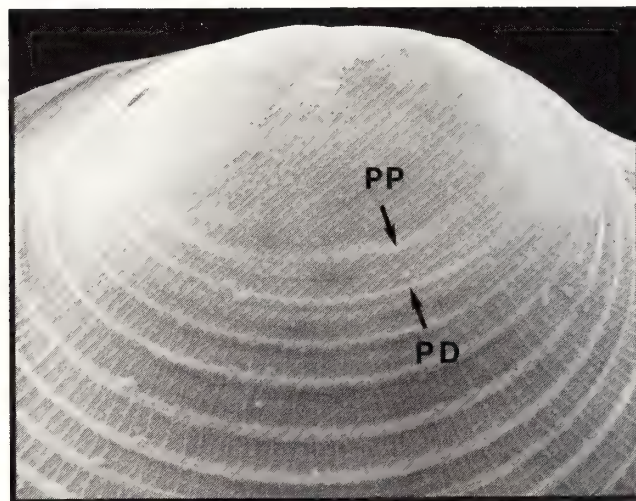


Fig. 3. Higher magnification of the specimen in figure 2 in the areas of transition from prodissoconch I to prodissoconch II (PP), and from prodissoconch II to dissoconch (PD).

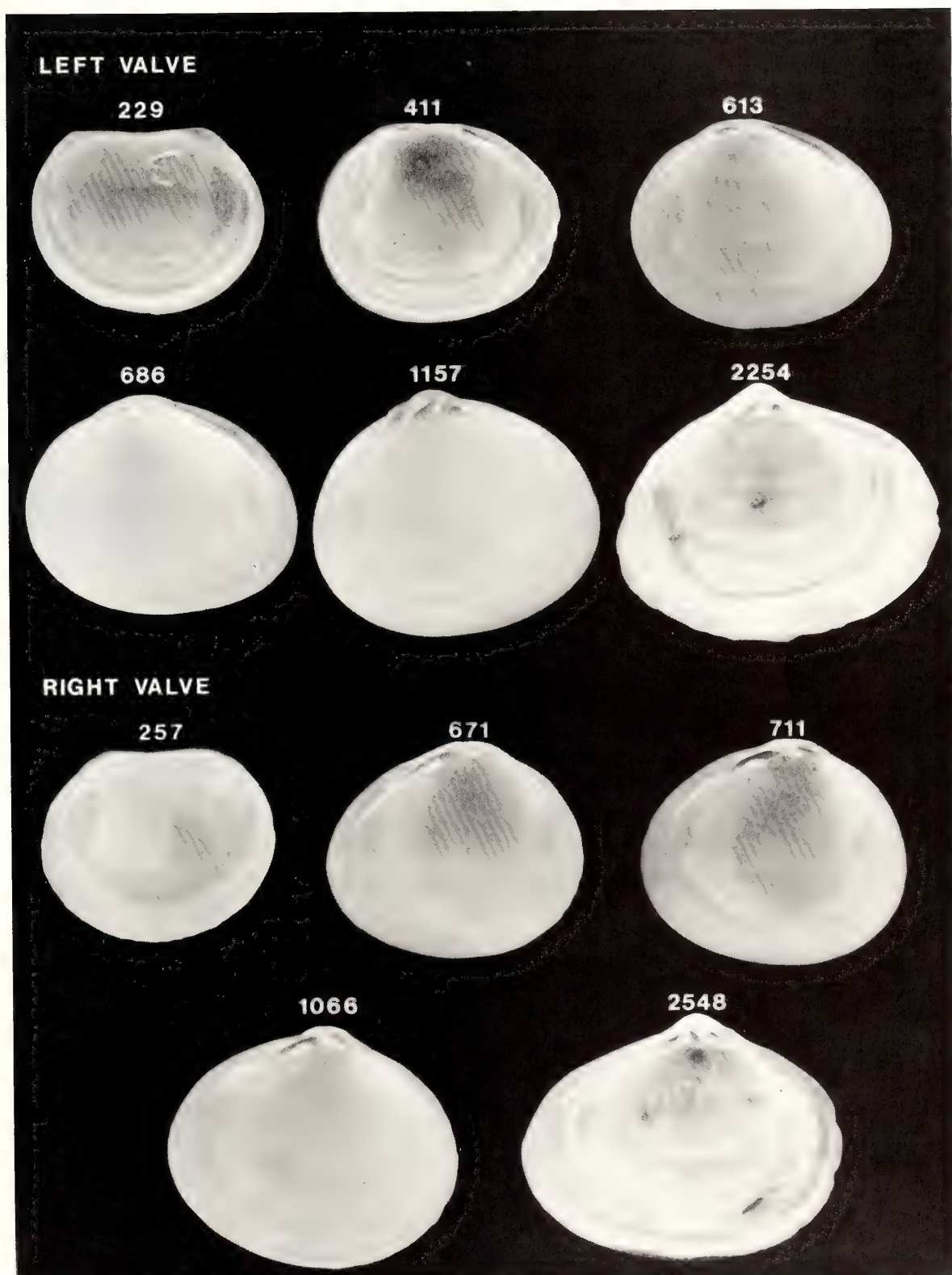


Fig. 4. Scanning electron micrographs of interior surfaces of disarticulated shell valves of young specimens of *Corbicula fluminea*. Numbers are shell length (μm). Specimens represent the Nassawango Creek, Maryland, population, except for the 2254 μm and 2548 μm animals, which were collected at Whites Ferry, Maryland.

because they were found only in shells larger than those released by the adults.

Tan Tiu and Prezant (1989) found tubules in their specimens to occur in a concentric band whose proximal border was about 700 μm from the hinge. We found tubules to occur closer to the hinge in our specimens; small numbers of tubules can be seen in the umbo region of some shells in figure 4 (left valve: 613 μm , 686 μm , 1157 μm ; right valve: 671 μm , 711 μm , 1066 μm ; see also the close-up views of some of these shells in figure 5). Tubules were fewer in number mm^{-2} in our specimens than reported by Tan Tiu and Prezant (1989). Our specimens are much smaller and presumably younger than those of Tan Tiu and Prezant (1989) so the few tubules that appear in the umbo region after release of young by adults may ultimately become covered as the shell enlarges and thickens with age, with the main proliferation of tubules occurring in older, larger individuals.

ACKNOWLEDGMENTS

New Jersey Agricultural Experiment Station Publication No. D-32402-1-90, supported by New Jersey State funds, NSF Grant EAR-84-17011, and various NOAA Sea Grant awards to Rutgers University. Contribution No. 2197 of the Center for Environmental and Estuarine Studies.

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Fig. 5. Scanning electron micrographs of the hinge apparatus of specimens pictured in figure 4. Arrowheads point toward ligament pit. Numbers are shell length (μm).

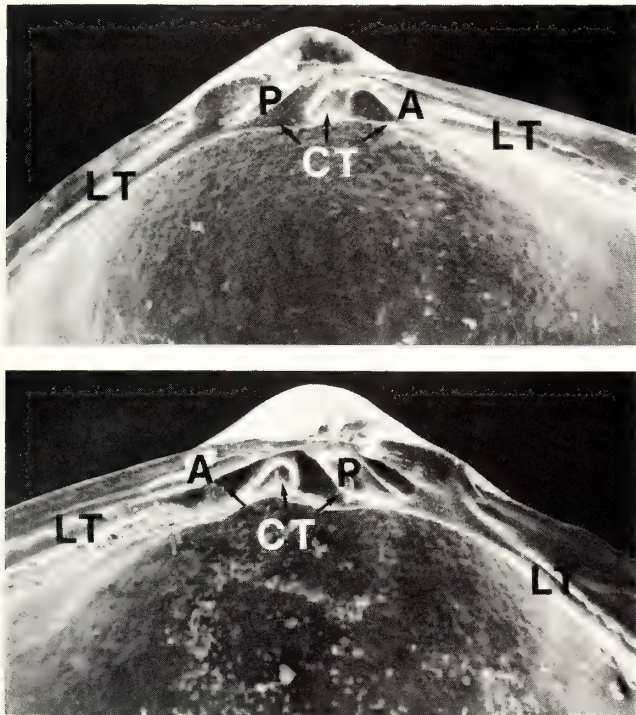


Fig. 6. Enlarged view of the hinge apparatus of (top) the left valve of the 2254 μm specimen of *Corbicula fluminea* and of (bottom) the right valve of the 2548 μm specimen in figure 4. [CT = cardinal teeth (A = anterior cardinal tooth, P = posterior cardinal tooth); LT = lateral tooth].

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Date of manuscript acceptance: 6 February 1991

A DISTRIBUTIONAL CHECKLIST OF THE FRESHWATER UNIONIDS (BIVALVIA:UNIONOIDEA) OF KENTUCKY

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ABSTRACT

A compilation of freshwater unionid records from Kentucky based on accepted literature reports, museum specimens, and personal collecting revealed that 103 taxa occur or occurred in the state. The fauna includes members of the Mississippian, Cumberlandian, and Atlantic Slope unionid regions. Eighteen taxa (17%) have been extirpated from the state or are extinct, and an additional 34 (33%) are rare in Kentucky or nationally. Significant new distributional information is presented, including the first Mississippi River record for each of 22 taxa and the first reported unionid bed from the lower Mississippi River.

Of the 297 freshwater unionid (mussel) (Bivalvia: Unionoidea) taxa recognized from the United States and Canada (Turgeon *et al.*, 1988), approximately 35% are known from Kentucky. Only Tennessee (Starnes and Bogan, 1988) and Alabama (J. D. Williams, pers. comm.) exceed Kentucky in unionid faunal richness.

Our knowledge of the highly diverse Kentucky unionid fauna began with the taxonomic studies of C. S. Rafinesque in 1818-1819. He described many species from the Ohio River and its Kentucky tributaries (Rafinesque, 1820, 1831), but considerable confusion and controversy surrounds the identity and nomenclature of many of these taxa (Bogan and Parmelee, 1983; Starnes and Bogan, 1988). Numerous studies followed that contributed significantly to our understanding of the distribution and zoogeographic relationships of the Kentucky fauna, including but not limited to Wilson and Clark (1914), Danglade (1922), Clench (1926), Ortmann (1926), van der Schalie (1939), Clench and van der Schalie (1944), Rosewater (1959), Neel and Allen (1964), Stansbery (1965,

1969), and Williams (1969). This historical information is complemented by a spate of recent (post-1969) distributional investigations (Table 1).

Even though a considerable body of literature exists regarding Kentucky unionids, only two compilations of distributional information on the fauna have been published, each with certain limitations. Bickel's (1967) checklist included 104 unionid taxa but was based solely on published records and contained no verified or new records. Schuster's (1988) update of Bickel's list included doubtful records, lacked much recent collection data, and did not identify the quality of collected specimens (e.g. live, subfossil, etc.). Moreover, neither effort assessed the distributional status of the fauna across the state. We attempt here to provide a comprehensive summary of the historical and current distribution of the Kentucky unionid fauna as judged from personal collecting, museum holdings, and accepted literature records covering the period from 1818 through 1987.

Table 1. Selected studies of Kentucky's freshwater unionid fauna published after 1969.

Drainage	Source
Ohio	Warren and Call, 1983; Stansbery and Cooney, 1985; Miller <i>et al.</i> , 1986; Williams and Schuster, 1989.
Tennessee	Chandler, 1982; Sickel and Chandler, 1982; Warren and Call, 1983; Pharris <i>et al.</i> , 1984; Sickel, 1985, 1987b.
Cumberland	Blankenship and Crockett, 1972; Harker <i>et al.</i> , 1980; Starnes and Starnes, 1980; Clarke, 1981, 1983; Call and Parmalee, 1981; Sickel, 1982, 1987a, 1988; Starnes and Bogan, 1982; DiStefano, 1984; Miller <i>et al.</i> , 1984; Thompson, 1985; Ahlstedt, 1986; Schuster <i>et al.</i> , 1989.
Tradewater	Harker <i>et al.</i> , 1981; Warren and Call, 1983.
Green	Clarke, 1981, 1983; Harker <i>et al.</i> , 1981; Taylor, 1983; Warren and Call, 1983.
Salt	Taylor, 1980a.
Kentucky	Williams, 1975; Houpp, 1980; Taylor, 1981, 1984; Tolin and King, 1986.
Licking	Taylor and Spurlock, 1983.
Kinniconick	Warren <i>et al.</i> , 1984.
Tygart	Zeto, 1979, 1980; Taylor, 1980b.
Big Sandy	Tolin and Schettig, 1984.

METHODS

Spot distribution maps were prepared for each taxon known from the state and were used to generate the distributional information reported here. The maps include locality information from accepted literature records, collections made by Kentucky State Nature Preserves Commission (KNP) personnel, and examination of specimens in the following institutional or personal collections (acronym in parentheses): Academy of Natural Sciences of Philadelphia (ANSP), Carnegie Museum (CM), Eastern Kentucky University (EKU), Field Museum of Natural History (FMNH), H. D. Athearn (HDA), Illinois Natural History Survey (INHS), Museum of Comparative Zoology (MCZ), Murray State University Museum of Zoology (MSUMZ), Marshall University (MU), National Museum of Natural History (NMNH), Ohio State University Museum of Zoology (OSUMZ), University of Illinois Museum of Natural History (UIMNH), and University of Michigan Museum of Zoology (UMMZ). Specimens collected by KNP are or will be deposited at OSUMZ. Each locality for a taxon was mapped as either a recent, historical, or archaeological record. Recent records include post-1969 collections of live or fresh-dead specimens; historical records include specimens collected prior to 1970; and archaeological records include shells taken during archaeological excavations. Specimens collected post-1969 that were weathered-dry and those reported in recent literature were mapped as historical records, unless the author(s) indicated or it was apparent that the specimens were collected alive or fresh-dead.

Annotations are presented alphabetically by genus and species within genus. Scientific and common names follow Turgeon *et al.* (1988). Cumberlandian endemics identified by

Ortmann (1924), Starnes and Bogan (1988), or Gordon and Layzer (1989) are indicated. Each annotation includes: 1) letters denoting the drainage(s) (Fig. 1) from which the taxon is known based on recent (Fig. 2) and historical and archaeological (Fig. 3) records; and 2) a brief statement regarding the known recent (post-1969) distribution. We use the terms "generally distributed", "occasional", and "sporadic" following the definitions of Smith (1965) (Table 2). For taxa that are rare in Kentucky (Warren *et al.*, 1986; United States Fish and Wildlife Service, 1989, 1990) or those known from only one or two recent collections in a drainage, we present the stream or drainage name, county of occurrence (Fig. 4), and source of the record(s). For collections made from the Ohio River in bordering states, we indicate the adjacent Kentucky county (Fig. 4). As appropriate, we include a statement indicating whether the taxon is believed to be extirpated in Kentucky or extinct. Finally, the current state conservation status as defined by the Kentucky Academy of Science-Kentucky State Nature Preserves Commission (KAS-KNP) (Warren *et al.*, 1986) is presented as E (= endangered), T (= threatened), or S (= special concern). Following the KAS-KNP status and a slash (/), the federal conservation status as defined by the United States Fish and Wildlife Service (USFWS) (1989, 1990) is abbreviated as E (= endangered), 2 (= candidate for listing), 3A (= persuasive evidence of extinction), 3B (= questionable specific status), and 3C (= more abundant or widely distributed than previously thought).

RESULTS

The following annotations document the occurrence and distribution of 103 unionid species in Kentucky (Table 3).

Actinonaias ligamentina (Lamarck, 1819). Mucket. B, E, F, G, H, J, K, L, N, O, Q, S. Generally distributed in the upper Green, Licking, and Big Sandy rivers. Occasional in the Ohio, upper Cumberland (below the Falls), and Kentucky rivers. Sporadic in the lower Green River. Rare in the Tennessee (below Kentucky Dam), Livingston/Marshall counties (Sickel, 1985), and Salt, Nelson County (KNP), rivers.

Actinonaias pectorosa (Conrad, 1834). Pheasantshell. Cumberlandian species. F, L, M. Generally distributed in the upper Cumberland River but sporadic above the Falls. Occasional in the Red River and Whippoorwill Creek of the lower Cumberland River.

Alasmidonta atropurpurea (Rafinesque, 1831). Cumberland elktoe. Cumberlandian species. L, M. Occasional

Table 2. Terminology and definitions regarding the current distribution for a particular unionid taxon in Kentucky (from Smith, 1965).

Generally distributed - implies that any suitable habitat within the prescribed area should be expected to yield specimens with a reasonably thorough search.

Occasional - implies that suitable-appearing habitat may or may not yield specimens even after prolonged search.

Sporadic - implies that encountering specimens of a given taxon cannot be predicted at all.

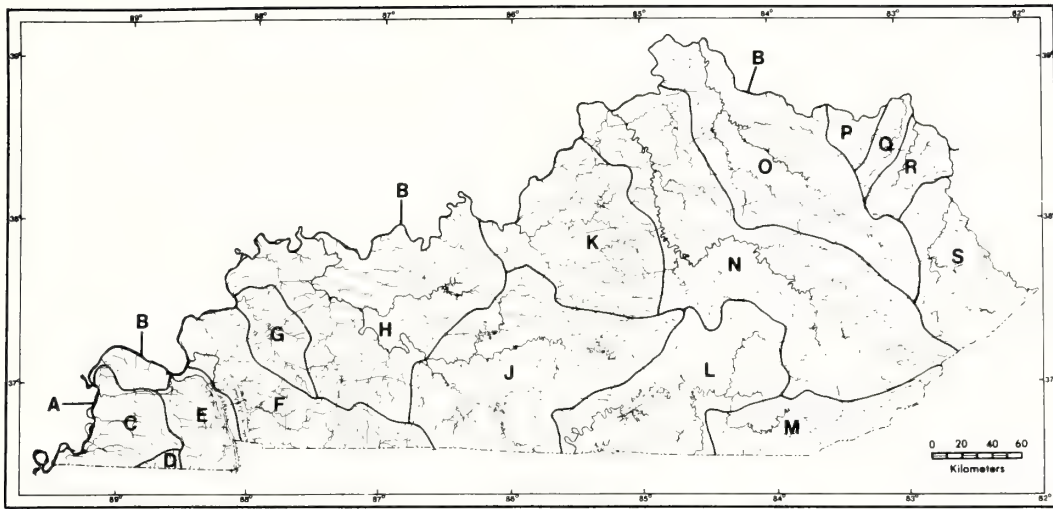


Fig. 1. Major river drainages in Kentucky: A, Mississippi River mainstream; B, Ohio River mainstream and minor tributaries; C, Mississippi River tributaries; D, Terrapin Creek and other small tributaries of Obion River; E, lower Tennessee and Clarks rivers; F, lower Cumberland River; G, Tradewater River; H, lower Green River; J, upper Green and Barren rivers; K, Salt River; L, upper Cumberland River below Cumberland Falls; M, upper Cumberland River above Cumberland Falls; N, Kentucky River; O, Licking River; P, Kinniconick and Salt Lick creeks; Q, Tygarts Creek; R, Little Sandy River; S, Big Sandy River.

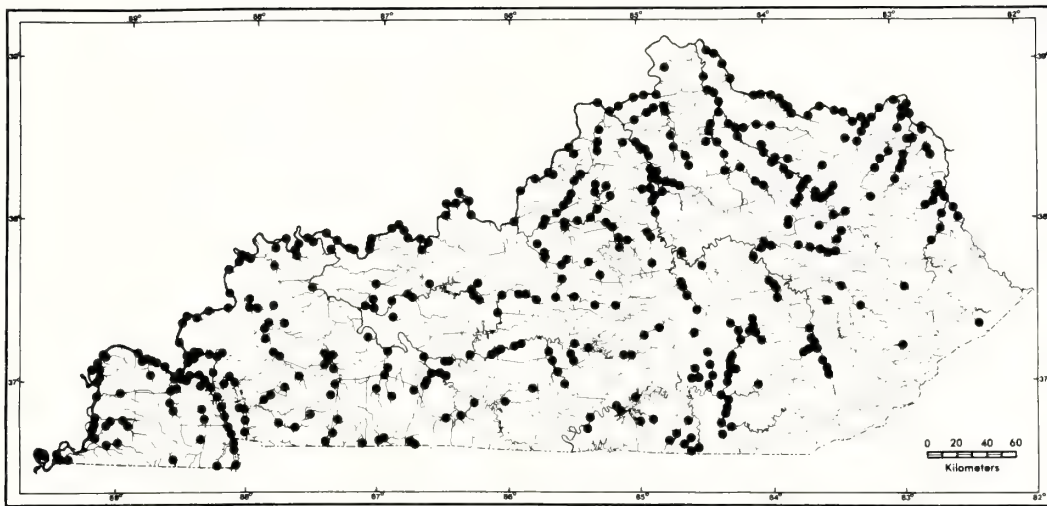


Fig. 2. Locations of unionid collections made in Kentucky after 1969 (each dot represents one or more collections).

and locally common in Marsh Creek (Call and Parmalee, 1981; KNP) and rare in Rock Creek (OSUMZ) (both McCreary County). The report from one locality in Horse Lick Creek, Jackson County (Ahlstedt, 1986), could represent a misidentification. E/2.

Alasmidonta marginata Say, 1818. Elktoe. B, F, J, K, L, M, N, O, R. Sporadic and rare in the upper Cumberland (below the Falls); Kentucky, where it is locally common; and Licking rivers. Known from a single record in the upper Green River, Hart County (KNP). T/-.

Alasmidonta viridis (Rafinesque, 1820). Slippershell mussel. B, F, H, J, K, L, M, N, O. Generally distributed in the Salt, Kentucky, and Licking rivers. Occasional and locally common in the upper Cumberland River (below the Falls) and

sporadic in the lower Cumberland River. Rare in Marsh Creek, McCreary County (Call and Parmalee, 1981; KNP).

Amblema plicata plicata (Say, 1817). Threeridge. A, B, E, F, G, H, J, K, L, N, O, P, Q, R, S. Generally distributed in the Ohio, Tennessee, lower Cumberland, upper Green, upper Cumberland (below the Falls), Kentucky, and Licking rivers. Occasional to sporadic in the lower Green, Salt, and Big Sandy rivers and Kinniconick and Tygarts creeks. Rare in the Mississippi River, Carlisle County (KNP), and Little Sandy River, Carter County (KNP).

Anodonta grandis Say, 1829. Giant floater. A, B, C, D, E, F, G, H, J, K, L, N, O, P, Q, R, S. Generally distributed in the Tennessee, Salt, Kentucky and Licking rivers but avoids the headwaters of the last two drainages. Occasional in

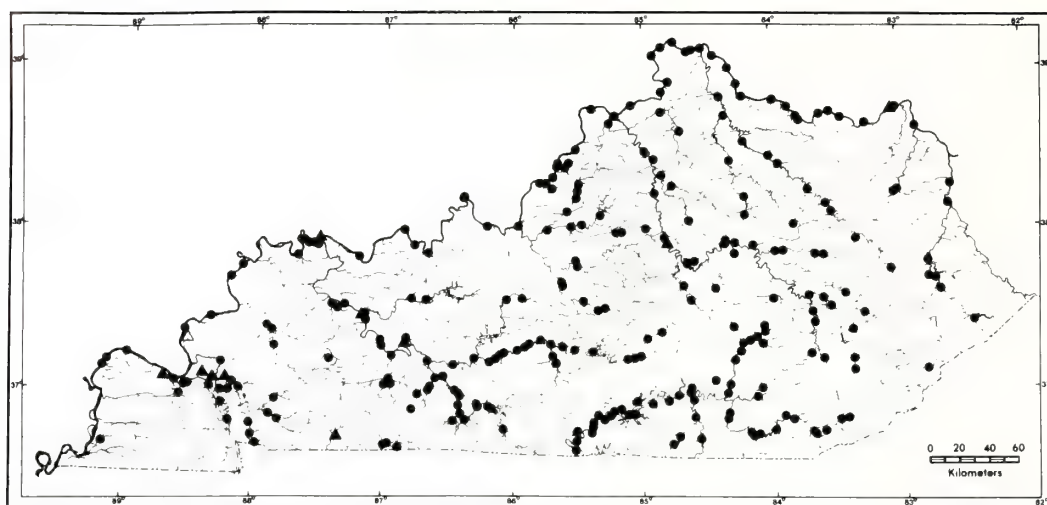


Fig. 3. Locations of unionid collections made in Kentucky before 1970. Dots represent historical collections (each dot represents one or more collections); triangles represent archaeological collections.

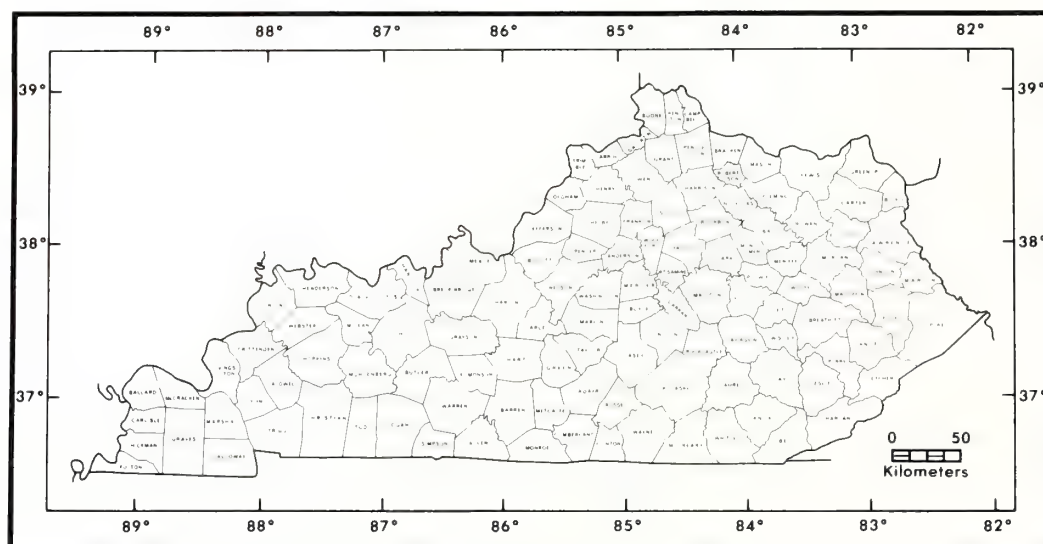


Fig. 4. The counties of Kentucky.

Mississippi River tributaries, and the Ohio, lower Cumberland, and Big Sandy rivers. Sporadic in the Mississippi, Tradewater, and Green rivers, and from Kinniconick Creek east to the Little Sandy River. Rare in the upper Cumberland River (below the Falls), McCreary/Wayne counties (Starnes and Bogan, 1982).

Anodonta imbecillis Say, 1829. Paper pondshell. A, B, C, E, F, G, H, J, K, L, N, O. Sporadic in the Ohio, Tennessee, Tradewater, Salt, upper Cumberland (below the Falls), and Kentucky rivers. Known from one or two records each from the Mississippi River, Carlisle County (KNP); Mayfield Creek, Carlisle County (KNP); lower Cumberland River, Trigg County (MSUMZ); lower Green River, Daviess and Muhlenberg counties (KNP); upper Green River, Barren (KNP) and Casey (W. R. Haag, pers. comm.) counties; and Licking River, Menifee

County (KNP).

Anodonta suborbiculata Say, 1831. Flat floater. B, C, E, F, H, J. Sporadic in Mississippi River tributaries and in impounded reaches of the Tennessee and lower Cumberland rivers. Rare in the Ohio River and minor tributaries, Ballard (KNP) and Campbell/Pendleton (Stansbery and Cooney, 1985) counties; lower Green River, Henderson County (KNP); and upper Green River, Edmonson County (KNP).

Anodontoides ferussacianus (Lea, 1834). Cylindrical papershell. B, F, G, H, J, K, L, M, N, O, Q. Sporadic and uncommon in the Salt and Licking rivers. Known from one or two records each in the lower Cumberland River, Christian County (KNP); Tradewater River, Christian County (KNP); lower Green River, Christian and Grayson counties (KNP); upper Cumberland River (above the Falls), McCreary County

(KNP); and Kentucky River, Owen County (MU).

Arcidens confragosus (Say, 1829). Rock-pocketbook. A, B, C, E, F, G, H, J. Occasional to sporadic in the lower Ohio, Tennessee, and lower Cumberland rivers. Rare in the Mississippi River, Carlisle and Hickman counties (KNP); Obion Creek, Hickman County (KNP); Tradewater River, Crittenden County (KNP); lower Green River, Butler and Ohio counties (KNP); and upper Green River, Warren County (KNP; OSUMZ).

Cumberlandia monodonta (Say, 1829). Spectaclecase. B, J, L. Formerly occurred in the Ohio (Call, 1900; MCZ; OSUMZ), upper Green (Stansbery, 1965), and upper Cumberland (below the Falls) (Neel and Allen, 1964) rivers. Known to persist in the upper Green River in Mammoth Cave National Park, Edmonson County (KNP), and in the upper Cumberland River based on one live specimen found below Wolf Creek Dam (Miller et al., 1984). E/2.

Cyclonaias tuberculata (Rafinesque, 1820). Purple wartyback. B, E, F, H, J, K, L, N, O, S. Generally distributed to occasional in the Ohio, upper Green, upper Cumberland (below the Falls), and Licking rivers. Occasional to sporadic in the Tennessee (below Kentucky Dam) and lower Cumberland rivers.

Cyprogenia stegaria (Rafinesque, 1820). Fanshell. B, E, F, H, J, K, L, N, O, Q, S. Occasional in the upper Green and lower Licking rivers. Rare in the Tennessee River (below Kentucky Dam), Livingston/Marshall counties (D. C. Wade, pers. comm.) and Tygarts Creek, Carter County (OSUMZ). T/E.

Dromus dromas (Lea, 1834). Dromedary pearlymussel. Cumberlandian species. E, F, L. Formerly occurred in the Tennessee and lower Cumberland rivers as judged from archaeological records (Casey, 1986) and in the upper Cumberland River (below the Falls) (Wilson and Clark, 1914; Neel and Allen, 1964). Considered extirpated. E/E.

Ellipsaria lineolata (Rafinesque, 1820). Butterfly. B, E, F, H, J, L, N, O. Generally distributed in the Ohio River and the Tennessee and lower Cumberland rivers (below the dams). Sporadic in the upper Green, where it is locally common, and Kentucky rivers. Known from one or two records each in the lower Green River, Butler County (KNP), and Licking River, Pendleton County (KNP).

Elliptio crassidens (Lamarck, 1819). Elephant-ear. A, B, E, F, G, H, J, K, L, N, O, P, Q, R, S. Generally distributed in the Ohio River. Occasional in the Tennessee and lower Cumberland rivers but avoids Kentucky and Barkley reservoirs. Sporadic in the upper Green, upper Cumberland (below the Falls), and Big Sandy rivers. Rare in the lower Green River, Butler and Ohio counties (KNP); Kinniconick Creek, Lewis County (Warren et al., 1984); Tygarts Creek, Greenup County (C. R. Burchett, Jr., pers. comm.); and Little Sandy River, Carter County (KNP).

Elliptio dilatata (Rafinesque, 1820). Spike. B, E, F, H, J, K, L, M, N, O, P, Q, R. Generally distributed in the upper Green, upper Cumberland (below the Falls), Kentucky, and Licking rivers, and Kinniconick Creek. Occasional in the Ohio, Tennessee, and lower Cumberland rivers but avoids Kentucky and Barkley reservoirs. Sporadic in Salt River and Tygarts Creek. Rare in the lower Green River, Ohio County (KNP);

upper Cumberland River (above the Falls), McCreary/Whitley counties (KNP); and Little Sandy River, Carter County (KNP).

Epioblasma arcaeiformis (Lea, 1831). Sugarspoon. Cumberlandian species. E, F, L. Archaeological records are available for the Tennessee and lower Cumberland rivers in Livingston and Lyon counties (Casey, 1986) and Todd County (OSUMZ). Also known historically from the Big South Fork Cumberland River, Pulaski County (Wilson and Clark, 1914). Presumed extinct (Stansbery, 1970, 1971). E/3A.

Epioblasma biemarginata (Lea, 1857). Angled riffleshell. Cumberlandian species. L. Known historically from the upper Cumberland River (below the Falls), Pulaski County (Johnson, 1978). Presumed extinct (Stansbery, 1971). E/3A.

Epioblasma brevidens (Lea, 1831). Cumberlandian combshell. Cumberlandian species. L. Known historically from the upper Cumberland River (below the Falls). Persists in Buck Creek, Pulaski County (EKU), and the Big South Fork Cumberland River, McCreary County (EKU; KNP), where it is occasional to sporadic. E/2.

Epioblasma capsaeformis (Lea, 1834). Oyster mussel. Cumberlandian species. L. Known historically from the upper Cumberland River (below the Falls). Presently rare in Buck Creek, Pulaski County (EKU), and Big South Fork Cumberland River, McCreary County (EKU; KNP). E/2.

Epioblasma flexuosa (Rafinesque, 1820). Leafshell. B, E, F, H, J, K, N. Known historically from the lower Ohio River (Goodrich and van der Schalie, 1944; KNP), and from the Ohio River adjacent Kenton (NMNH; OSUMZ; UMMZ) and Campbell (OSUMZ) counties. Known from prehistoric sites or subfossil specimens from the Ohio River, Henderson (Parmalee, 1960), Ballard (KNP), and Livingston (MSUMZ) counties; Tennessee River, Livingston/Marshall counties (Casey, 1986); and Cumberland River, Livingston/Lyon (Casey, 1986) and Todd (OSUMZ) counties. Described by Rafinesque (1820) from the Green, Salt, and Kentucky rivers; Johnson (1980) regarded these records as spurious. Presumed extinct (Stansbery, 1970, 1971).

Epioblasma florentina florentina (Lea, 1857). Yellow blossom. Cumberlandian species. L. Formerly occurred in the upper Cumberland River (below the Falls), Cumberland, Pulaski, and Russell counties (Wilson and Clark, 1914; Neel and Allen, 1964; Johnson, 1978). An archaeological site specimen (Todd County; OSUMZ) could represent this taxon or *E. florentina walkeri*. Considered extirpated. E/E.

Epioblasma florentina walkeri (Wilson and H. W. Clark, 1914). Tan riffleshell. Cumberlandian species. L. Historically known from the upper Cumberland River (below the Falls), Pulaski and Russell counties (Neel and Allen, 1964; Stansbery, 1976a). See *E. florentina florentina* annotation. Probably extirpated. E/E.

Epioblasma haysiana (Lea, 1833). Acornshell. Cumberlandian species. L. Formerly known from the upper Cumberland River (below the Falls) (Wilson and Clark, 1914; Neel and Allen, 1964; UMMZ). Considered extirpated. E/3A.

Epioblasma lewisii (Walker, 1910). Forkshell. Cumberlandian species. B, L. Formerly occurred in the Ohio River, Kenton County (OSUMZ), and the upper Cumberland River (below the Falls), Pulaski and Russell counties (Walker, 1910;

Neel and Allen, 1964). Presumed extinct (Stansbery, 1970, 1971). E/3A.

Epioblasma obliquata obliquata (Rafinesque, 1820). Catspaw. B, H, J, L, N, O. Known historically from the Ohio River adjacent Indiana (Call, 1900; Goodrich and van der Schalie, 1944) and Kenton County (Johnson, 1978), and at archaeological sites adjacent Henderson (Parmalee, 1960) and Lewis (OSUMZ) counties. Also formerly occurred in the lower Green River, Butler County (Clench and van der Schalie, 1944; Johnson, 1978); upper Green River, Hart (EKU) and Warren (OSUMZ) counties; upper Cumberland River (below the Falls), Cumberland (Neel and Allen, 1964), Pulaski (Johnson, 1978), and Russell (EKU) counties; Kentucky River (Rafinesque, 1820); and Licking River (Johnson, 1978, 1980; MCZ). Persists only in the Green River (Stansbery, 1971). E/E.

Epioblasma obliquata perobliqua (Conrad, 1836). White catspaw. B. This federally endangered unionid (as *E. sulcata delicata*) could have occurred in the lower Ohio River (Hoggarth, 1990) but is presumed extirpated. -/E.

Epioblasma personata (Say, 1829). Round combshell. B. Known only from the Ohio River at Cincinnati, Kenton County (Stansbery, 1971; Johnson, 1978). Presumed extinct (Stansbery, 1970, 1971). -/3A.

Epioblasma propinqua (Lea, 1857). Tennessee riffleshell. B. Known only from the Ohio River at Cincinnati, Kenton County (Johnson, 1978). Presumed extinct (Stansbery, 1970, 1971). -/3A.

Epioblasma sampsonii (Lea, 1861). Wabash riffleshell. B, H, N. Known historically from the Ohio River at Cincinnati, Kenton County (Johnson, 1978). Also collected from archaeological sites in the Ohio River, Henderson County (Parmalee, 1960); lower Green River, McLean County (D. H. Stansbery, pers. comm.); and the Kentucky River, Woodford County (Call and Robinson, 1983; D. H. Stansbery, pers. comm.). Presumed extinct (Stansbery, 1970, 1971; United States Fish and Wildlife Service, 1984).

Epioblasma stewardsoni (Lea, 1852). Cumberland leafshell. Cumberlandian species. L. Known only from the upper Cumberland River (below the Falls), Pulaski County (Johnson, 1978). Presumed extinct (Stansbery, 1970, 1971). E/3A.

Epioblasma torulosa rangiana (Lea, 1839). Northern riffleshell. B, H, J, K, N, O. Known historically from the Ohio River, Kenton County (MCZ); upper Green River, Edmonson (Ortmann, 1926; KNP), Green (MCZ; UMMZ), Hart (Stansbery, 1965; KNP; OSUMZ), Taylor (MCZ), and Warren (MCZ; OSUMZ; UMMZ) counties; Salt River, Nelson (KNP; OSUMZ) and Spencer (OSUMZ) counties; Kentucky River, Franklin County (KNP; OSUMZ); and Licking River, Bath (KNP; OSUMZ) and Pendleton (OSUMZ) counties. Known from archaeological sites in the lower Green, Butler County (Patch, 1976), and Kentucky, Woodford County (Call and Robinson, 1983; D. H. Stansbery, pers. comm.) rivers. As judged from recently collected fresh-dead specimens, persists only in the upper Green River (KNP; OSUMZ). E/2.

Epioblasma torulosa torulosa (Rafinesque, 1820). Tubercled blossom. B, E, F, J, K, O. Present historically in the Ohio River, Kenton (MCZ; OSUMZ) and McCracken (Parma-

lee, 1967) counties; lower Tennessee River (D. H. Stansbery, pers. comm.); lower Cumberland River, Livingston County (MSUMZ); upper Green River, Warren County (Johnson, 1978; KNP; MCZ); Salt River, Nelson County (KNP; OSUMZ); and Licking River, Pendleton County (OSUMZ). Prehistoric records are available for the Ohio River adjacent Henderson (Parmalee, 1960) and Lewis/Greenup (OSUMZ) counties. Also may have occurred in the Kentucky River (Rafinesque, 1820; Dangle, 1922). Possibly extirpated. E/E.

Epioblasma triquetra (Rafinesque, 1820). Snuffbox. B, F, H, J, K, L, N, O, P, Q. Occasional in the middle Licking River. Sporadic in the upper Green, upper Cumberland (below the Falls), and upper Kentucky rivers and Kinniconick and Tygarts creeks. S/-.

Fusconaia ebena (Lea, 1831). Ebonyshell. A, B, E, F, J, L, O. Generally distributed and common in the Ohio, lower Tennessee, and lower Cumberland rivers; rare in the Mississippi River, Hickman County (KNP).

Fusconaia flava (Rafinesque, 1820). Wabash pigtoe. B, E, F, G, H, J, K, L, N, O, P, Q, R, S. Generally distributed in the Ohio and Licking rivers. Occasional to sporadic in the Tennessee, lower Cumberland, lower and upper Green, Salt, Kentucky, Little Sandy, and Big Sandy rivers and Kinniconick and Tygarts creeks.

Fusconaia subrotunda (Lea, 1831). Long-solid. B, E, F, H, J, K, L, N, O, S. Sporadic and rare in the Tennessee, upper Green, Licking, and Big Sandy rivers. T/-.

Glebulula rotundata (Lamarck, 1819). Round pearlshell. B. Known from a single collection from the Ohio River at Louisville, Jefferson County (OSUMZ).

Hemistena lata (Rafinesque, 1820). Cracking pearlymussel. B, J, L, N. Known historically from the Ohio River, Jefferson (NMNH), Carroll (OSUMZ), and Kenton (OSUMZ) counties; the upper Green River, Edmonson (MCZ) and Hart (Clench, 1926; Ortmann, 1926; Stansbery, 1965) counties; the upper Cumberland River (below the Falls) (Wilson and Clark, 1914; Neel and Allen, 1964); and the Kentucky River (Rafinesque, 1820). Could persist in the upper Green River. E/E.

Lampsilis abrupta (Say, 1831.) Pink mucket. B, E, F, J, K, L. Sporadic and rare in the lower Ohio River (United States Fish and Wildlife Service, 1985); Tennessee River (below Kentucky Dam), Livingston/Marshall counties (Sickel, 1985, 1987b; United States Fish and Wildlife Service, 1985); and upper Green River, Edmonson, Hart, and Warren counties (KNP; OSUMZ). E/E.

Lampsilis cardium (Rafinesque, 1820). Plain pocketbook. B, E, F, G, H, J, K, L, M, N, O, P, Q, R, S. Generally distributed in the upper Cumberland (below the Falls), Kentucky, and Licking rivers and Kinniconick Creek. Occasional in the upper Green River, Tygarts Creek, and Big Sandy River. Sporadic in the Ohio, lower Green, Salt, and upper Cumberland (above the Falls) rivers. Known from one or two collections each in the Tennessee River, McCracken County (KNP); lower Cumberland River, Livingston and Logan counties (Sickel, 1987a; KNP); and Little Sandy River, Carter County (KNP).

Lampsilis fasciola Rafinesque, 1820. Wavy-rayed

lampmussel. B, F, J, K, L, M, N, O, P, Q. Generally distributed in the upper Cumberland River (below the Falls). Occasional in the Kentucky River; sporadic in the lower Cumberland, upper Green, upper Cumberland (above the Falls), and Licking rivers. Rare in Kinniconick Creek, Lewis County (Warren *et al.*, 1984).

Lampsilis ovata (Say, 1817). Pocketbook. B, E, F, H, J, K, L, M. Occasional and locally common in the upper Green River. Rare in the lower Ohio River, Ballard County (Miller *et al.*, 1986), and the Tennessee River, Livingston/Marshall counties (Sickel, 1985, 1987b). Wilson and Clark (1914) were told that *L. ovata* was transplanted into the upper Cumberland River above the Falls. E/-.

Lampsilis siliquoidea (Barnes, 1823). Fatmucket. B, C, E, F, G, H, J, K, N, O, P, Q, R, S. Generally distributed from the upper Green River east to the Little Sandy River. Occasional in the lower Green and Big Sandy rivers. Rare in the Ohio River, Campbell County (W. R. Haag, pers. comm.); Obion Creek, Hickman County (KNP); and lower Cumberland River, Christian and Trigg counties (KNP).

Lampsilis teres (Rafinesque, 1820). Yellow sandshell. A, B, C, E, F, G, H, J, K, N, O. Sporadic in the Ohio, Tennessee, upper Green, and Kentucky rivers. Rare in the Mississippi River and tributaries, Carlisle (KNP) and Hickman counties (KNP; NMNH); lower Green River, Butler/Muhlenberg and Ohio counties (KNP); Salt River, Spencer County (KNP); and Licking River, Bath (KNP) and Pendleton (OSUMZ) counties.

Lasmigona complanata complanata (Barnes, 1823). White heelsplitter. A, B, C, E, F, G, H, J, K, N, O, R, S. Occasional in the Salt and Licking rivers; sporadic in the Tennessee, lower Cumberland, lower Green, and Kentucky rivers. Rare in the Mississippi River, Hickman County (KNP); Obion Creek, Hickman County (KNP); Ohio River, McCracken County (EKU); Tradewater River, Crittenden County (KNP); upper Green River, Warren County (MSUMZ; OSUMZ); and Big Sandy River, Lawrence County (Tolin and Schettig, 1984).

Lasmigona compressa (Lea, 1829). Creek heelsplitter. B, Q, R. Rare in Tygarts Creek, Carter and Greenup counties (Zeto, 1979; Taylor, 1980b), and the Little Sandy River, Carter County (OSUMZ). T/-.

Lasmigona costata (Rafinesque, 1820). Fluted-shell. B, F, G, H, J, K, L, N, O, P, Q, R, S. Generally distributed in the upper Cumberland (below the Falls) and Licking rivers. Occasional in the upper Green, Salt, Kentucky, and Big Sandy rivers; sporadic in Tygarts Creek. Known from one or two localities each in the Ohio River, Campbell/Pendleton counties (Stansbery and Cooney, 1985); lower Cumberland River, Logan County (KNP); Kinniconick Creek, Lewis County (Warren *et al.*, 1984); and Little Sandy River, Carter County (KNP; MU).

Lasmigona subviridis (Conrad, 1835). Green floater. Q. Known only from a single collection from Tygarts Creek, Greenup County (Zeto, 1979, 1980). T/-.

Leptodea fragilis (Rafinesque, 1820). Fragile papershell. A, B, C, E, F, G, H, J, K, L, N, O, P, Q, R, S. Generally distributed to occasional in the Ohio, Tennessee, upper Cumberland (below the Falls), Licking, and Big Sandy rivers. Sporadic in the Mississippi River and tributaries, lower

Cumberland, upper Green, Salt, Kentucky, and Little Sandy rivers and Tygarts Creek. Rare in the Tradewater River, Crittenden/Webster counties (KNP); lower Green River, Butler and Ohio counties (KNP); and Kinniconick Creek, Lewis County (Warren *et al.*, 1984).

Leptodea leptodon (Rafinesque, 1820). Scaleshell. B, J, L, N. Formerly known from the Ohio River adjacent Indiana (Goodrich and van der Schalie, 1944), near Cincinnati, Kenton County (Stansbery, 1970), and at Constance, Boone County (CM). Also occurred in the lower Ohio (Rafinesque, 1820) and Kentucky (Vanatta, 1915) rivers. Last collected from the upper Green River, Hart County (Stansbery, 1965), and the upper Cumberland River (below the Falls), Cumberland and Russell counties (Wilson and Clark, 1914; Neel and Allen, 1964). Possibly extirpated. E/2.

Lexingtonia dolabelloides (Lea, 1840). Slabside pearlymussel. Cumberlandian species. F. Discovered recently in the Red River, Logan County (KNP; MSUMZ). Possibly extirpated. -/2.

Ligumia recta (Lamarck, 1819). Black sandshell. A, B, E, F, H, J, K, L, N, O, Q, S. Occasional in the upper Green and upper Cumberland (below the Falls) rivers; sporadic in the Ohio and Tennessee rivers. Rare in the Mississippi River, Hickman County (KNP), lower Cumberland River, Livingston/Lyon counties (Sickel, 1982; MSUMZ); lower Green River, Butler County (KNP); Kentucky River, Henry/Owen counties (Tolin and King, 1986); Licking River, Pendleton County (KNP); Tygarts Creek, Greenup County (C. R. Burchett, Jr., pers. comm.); and Big Sandy River, Lawrence County (Tolin and Schettig, 1984).

Ligumia subrostrata (Say, 1831). Pondmussel. B, C, E, G, H. Sporadic in the Tennessee River; rare in an Ohio River floodplain lake, McCracken County (KNP); and the Tradewater River, Hopkins County (Warren and Call, 1983).

Medionidus conradicus (Lea, 1834). Cumberland moccasinshell. Cumberlandian species. F, L. Generally distributed to occasional in the upper Cumberland River (below the Falls). Known only from Red River, Logan County, in the lower Cumberland River (KNP; OSUMZ).

Megalania nervosa (Rafinesque, 1820). Washboard. A, B, E, F, G, H, J, K, L, N, O, Q, S. Generally distributed in the Tennessee and lower Cumberland (below Barkley Dam) rivers. Occasional in the Ohio, upper Green, Kentucky, and Licking rivers. Sporadic in the lower Green and Salt rivers. Rare in the Mississippi River, Fulton and Hickman counties (KNP); Tradewater River, Crittenden County (KNP); Tygarts Creek, Greenup County (W. R. Haag, pers. comm.); and Big Sandy River, Lawrence County (Tolin and Schettig, 1984).

Obliquaria reflexa Rafinesque, 1820. Threehorn wartyback. A, B, E, F, G, H, J, K, L, N, O. Generally distributed in the Ohio and Tennessee rivers. Occasional in the lower Cumberland, upper Green, and Licking rivers. Sporadic in the lower Green and Kentucky rivers. Known from single localities in the Mississippi River, Hickman County (KNP); and Salt River, Spencer County (OSUMZ).

Obovaria olivaria (Rafinesque, 1820). Hickorynut. A, B, E, F, H, J, L, N. Generally distributed and common in the Ohio River. Rare in the Mississippi River, Hickman County (KNP);

Tennessee River, Livingston/Marshall counties (MSUMZ); and Kentucky River (Williams, 1975).

Obovaria retusa (Lamarck, 1819). Ring pink. B, E, F, H, J, L, N. Persists only in the Tennessee River (below Kentucky Dam), Livingston/Marshall counties (Sickel, 1985), and upper Green River, Edmonson and Hart counties (Stansbery, 1965; KNP). E/E.

Obovaria subrotunda (Rafinesque, 1820). Round hickorynut. B, E, F, H, J, K, L, N, O, Q, R, S. Occasional in the upper Cumberland (below the Falls) and Big Sandy rivers. Sporadic in the upper Green, Kentucky, and Licking rivers and Tygarts Creek. Rare in the Ohio River, Campbell/Pendleton counties (EKU); lower Cumberland River, Logan County (KNP); and Little Sandy River, Boyd County (MU).

Pegias fabula (Lea, 1838). Little-wing pearlymussel. Cumberlandian species. F, L. Rare in Horse Lick Creek, Jackson County (DiStefano, 1984); Kennedy Creek, Wayne County (KNP); Little South Fork Cumberland River, McCreary/Wayne counties (Starnes and Starnes, 1980; Starnes and Bogan, 1982); Big South Fork Cumberland River, McCreary County (Ahlstedt, 1986); and Whippoorwill Creek, Logan County (KNP). Known from one live specimen from Buck Creek, Pulaski County (Stansbery, 1976b; OSUMZ). E/E.

Plectomerus dombeyanus (Valenciennes, 1827). Bankclimber. B, E. Known only from the Tennessee River (Kentucky Lake), Trigg County (Chandler, 1982; Pharris *et al.*, 1984).

Plethobasus cicatricosus (Say, 1829). White wartyback. B, F. Formerly known from the Ohio River, Kenton (NMNH; UMMZ) and Carroll (OSUMZ) counties. Also known from archaeological sites along the Ohio River in Henderson (Parmalee, 1960), Livingston (Casey, 1986), and Oldham (KNP) counties; and in the lower Cumberland River in Livingston/Lyon (Casey, 1986) and Todd (OSUMZ) counties. Possibly extirpated. E/E.

Plethobasus cooperianus (Lea, 1834). Orange-foot pimpleback. B, E, F, H, J, K, L. Rare and only known to persist in the Ohio River, Ballard County (Miller *et al.*, 1986; Williams and Schuster, 1989), and lower Tennessee River, Livingston/Marshall counties (Sickel, 1985; MSUMZ). E/E.

Plethobasus cyphus (Rafinesque, 1820). Sheepnose. B, E, F, H, J, L, O. Generally distributed and common in the Ohio River. Occasional in the upper Green River; sporadic in the Tennessee (below Kentucky Dam) and Licking rivers. S/-.

Pleurobema clava (Lamarck, 1819). Clubshell. B, E, F, J, K, N, O. As judged from recently collected fresh-dead specimens, probably persists in the upper Green River (KNP; OSUMZ). E/2.

Pleurobema coccineum (Conrad, 1834). Round pigtoe. B, E, F, H, J, K, L, N, O, Q. Occasional in the upper Green and upper Cumberland (below the Falls) rivers. Sporadic in the Ohio, Kentucky, and Licking rivers. Rare in the lower Tennessee River, Livingston/Marshall counties (MSUMZ).

Pleurobema cordatum (Rafinesque, 1820). Ohio pigtoe. B, E, F, H, J, K, L, N, O, Q. Generally distributed in the Ohio River. Occasional in the Tennessee and lower Cumberland (below the dams), upper Green, and Kentucky rivers. Known from one record each in the lower Green River, Butler County

(KNP), and Tygarts Creek, Greenup County (W. R. Haag, pers. comm.).

Pleurobema oviforme (Conrad, 1834). Tennessee clubshell. Cumberlandian species. F, L. Sporadic and rare in the lower and upper Cumberland River (below the Falls). Persists in Big South Fork Cumberland River, McCreary County (KNP); Buck Creek, Pulaski County (Schuster *et al.*, 1989); Horse Lick Creek, Jackson/Rockcastle counties (KNP); Kennedy Creek, Wayne County (Ahlstedt, 1986); Little South Fork Cumberland River, McCreary/Wayne counties (Starnes and Bogan, 1982; Ahlstedt, 1986); and Whippoorwill Creek, Logan County (KNP). E/2.

Pleurobema plenum (Lea, 1840). Rough pigtoe. B, H, J, L, N, O. Sporadic and rare in the upper Green River, Butler, Edmonson, Hart, and Warren counties (KNP, OSUMZ). E/E.

Pleurobema pyramidatum (Lea, 1840). Pyramid pigtoe. B, E, F, H, J, K, L, N, O. Sporadic in the upper Green River, Butler, Edmonson, Hart, and Warren counties (KNP); rare in the Tennessee River, Livingston/Marshall counties (MSUMZ). E/3B.

Potamilus alatus (Say, 1817). Pink heelsplitter. A, B, C, E, F, G, H, J, K, L, N, O, P, Q, R, S. Generally distributed to occasional throughout the state but sporadic in the Mississippi River. Known from single collections in Obion Creek, Hickman County (KNP), and Tradewater River, Crittenden County (KNP).

Potamilus capax (Green, 1832). Fat pocketbook. B, F, H. Sporadic and rare in the Ohio River from the Wabash River, Union County, downstream to Ballard County (KNP), and the extreme lower Cumberland River, Livingston County (Sickel, 1987a). E/E.

Potamilus ohioensis (Rafinesque, 1820). Pink papershell. A, B, C, E, F, H, J, K, L, N, O, S. Generally distributed to occasional in the Mississippi and lower Ohio rivers upstream to the Wabash River. Sporadic in the Tennessee and lower Cumberland rivers. Known from one or two collections each in Obion Creek, Hickman County (KNP); lower Green River, Butler County (KNP); upper Cumberland River (below the Falls), Laurel (KNP) and Pulaski (EKU) counties; Kentucky River, Franklin and Henry/Owen counties (Tolin and King, 1986); and Big Sandy River, Lawrence County (Tolin and Schettig, 1984).

Potamilus purpuratus (Lamarck, 1819). Bleufer. A, B. Known only from two specimens from the Mississippi River, Fulton County (KNP), and one specimen from an Ohio River oxbow lake, Ballard County (OSUMZ).

Ptychobranhus fasciolaris (Rafinesque, 1820). Kidneyshell. B, E, F, H, J, K, L, N, O, P, Q, R, S. Generally distributed to occasional from the upper Green River east to Tygarts Creek. Occasional in the lower Cumberland River. Rare in the Ohio River, Lewis County (EKU); Tennessee River, Livingston/Marshall counties (MSUMZ); Little Sandy River, Carter (KNP) and Greenup (OSUMZ) counties; and Big Sandy River, Floyd (OSUMZ) and Lawrence (Tolin and Schettig, 1984) counties.

Ptychobranhus subtentum (Say, 1825). Fluted kidneyshell. Cumberlandian species. F, L. Occasional in the upper Cumberland River (below the Falls). T/-.

Quadrula apiculata (Say, 1829). Southern mapleleaf. A, B, E, H, J. Sporadic in the lower Ohio and Tennessee rivers. Known from one record each in the Mississippi River, Hickman County (KNP), and lower Green River, Butler County (KNP).

Quadrula cylindrica cylindrica (Say, 1817). Rabbitsfoot. B, E, F, H, J, K, L, N, O, S. Sporadic in the lower Ohio River, Henderson (Williams and Schuster, 1989) and McCracken (INHS) counties; Tennessee River (below Kentucky Dam), Livingston/Marshall counties (Sickel, 1985); lower Cumberland River, Logan County (KNP); and upper Green River, Green, Hart, and Taylor counties (KNP; OSUMZ). E/-.

Quadrula fragosa (Conrad, 1835). Winged mapleleaf. B, F. Known historically from the Ohio River, Oldham (Call, 1900) and Kenton (LaRocque, 1967) counties; and the lower Cumberland River, Lyon and Trigg counties (Wilson and Clark, 1914). A recently collected fresh-dead specimen from the lower Ohio River, Ballard County (KNP; OSUMZ), could represent this or an undescribed species (D. H. Stansbery, pers. comm.). E/3C.

Quadrula metanevra (Rafinesque, 1820). Monkeyface. B, E, F, H, J, L, N, O. Generally distributed to occasional in the Ohio, Tennessee and lower Cumberland (below the dams), and Licking rivers. Sporadic in the upper Green River. Known from a single record in the Kentucky River, Henry/Owen counties (Tolin and King, 1986).

Quadrula nodulata (Rafinesque, 1820). Wartyback. A, B, E, F, G, H, K, N, O. Generally distributed in the Ohio and Tennessee rivers. Occasional in the lower Cumberland, lower Kentucky, and Licking rivers. Rare in the Mississippi River, Carlisle and Hickman counties (KNP); Tradewater River, Crittenden County (KNP); lower Green River, Ohio County (KNP); and Salt River, Spencer County (KNP).

Quadrula pustulosa pustulosa (Lea, 1831). Pimpleback. A, B, C, E, F, G, H, J, K, L, N, O, P, Q, R, S. Generally distributed in the Ohio, Tennessee, upper Green, upper Cumberland (below the Falls), and Licking rivers. Occasional to sporadic in the lower Cumberland (below Barkley Dam), lower Green, Salt, Kentucky, and Big Sandy rivers and Tygarts Creek. Known only from one or two collections each in the Mississippi River, Hickman County (KNP); Obion Creek, Hickman County (KNP); Kinniconick Creek, Lewis County (Warren *et al.*, 1984); and Little Sandy River, Carter County (KNP).

Quadrula quadrula (Rafinesque, 1820). Mapleleaf. A, B, C, E, F, G, H, J, K, L, N, O, Q, R, S. Generally distributed in the Ohio, Tennessee, and lower Cumberland rivers. Occasional to sporadic in the Mississippi, lower Green, upper Green, Salt, Kentucky, Licking, Little Sandy, and Big Sandy rivers. Rare in Obion Creek, Hickman County (KNP), and Tradewater River, Crittenden County (KNP).

Quadrula sparsa (Lea, 1841). Appalachian monkeyface. Cumberlandian species. L. Ortmann (1912) examined two specimens from the upper Cumberland River (below the Falls), Cumberland and Pulaski counties; these records are included provisionally, but the specimens apparently are not extant. Neither Starnes and Bogan (1988) nor Gordon and Layzer (1989) recognized either *sparsa* or *intermedia* as

occurring in the Cumberland River, and the specimens reported by Ortmann (1912) could have been based on *tuberosa*. E/E.

Quadrula tuberosa (Lea, 1840). Rough rockshell. Cumberlandian species. L. Formerly occurred in the upper Cumberland River (below the Falls) (Wilson and Clark, 1914; UIMNH; UMMZ). Presumed extinct (Ahlfstedt, 1983).

Simpsonaias ambigua (Say, 1825). Salamander mussel. B, J, K, N, O, P, Q, R. Occasional to sporadic in the Kentucky, Licking, and Little Sandy rivers and Tygarts Creek. Known only from two records in Kinniconick Creek, Lewis County (Warren *et al.*, 1984). T/2.

Strophitus undulatus (Say, 1817). Squawfoot. B, F, H, J, K, L, M, N, O, P, Q, R, S. Occasional to sporadic from the upper Green River east to the Big Sandy River; unknown from recent collections in the upper Cumberland River (above the Falls).

Toxolasma lividus (Rafinesque, 1831). Purple lilliput. B, F, H, J, K, L. Sporadic and rare in the upper Cumberland River (below the Falls). Known from a single recent collection in the lower Cumberland River, Todd County (OSUMZ). E/2.

Toxolasma parvus (Barnes, 1823). Lilliput. B, C, E, H, J, K, L, M, N, O, R. Sporadic in the Ohio, lower Green, and Salt rivers. Known from one or two records each in Mississippi River tributaries, Fulton County (KNP); Tennessee River (Kentucky Reservoir), Calloway/Trigg and Livingston/Marshall counties (Chandler, 1982); and Little Sandy River, Greenup County (MSUMZ).

Toxolasma texasensis (Lea, 1857). Texas lilliput. E. G. Known only from Cypress Creek, Marshall County (KNP); Smith Ditch, Union County (Warren and Call, 1983); and Tradewater River, Hopkins County (KNP).

Tritogonia verrucosa (Rafinesque, 1820). Pistolgrip. A, B, C, E, F, G, H, J, K, L, N, O, P, Q, R, S. Generally distributed to occasional in the Ohio, Tennessee, lower Cumberland, lower and upper Green, Salt, upper Cumberland (below the Falls), Kentucky, Licking, and Big Sandy rivers and Kinniconick Creek. Sporadic in Tygarts Creek and Little Sandy River. Known from one location each in the Mississippi River and Obion Creek, both Hickman County (KNP).

Truncilla donaciformis (Lea, 1828). Fawnsfoot. B, E, F, G, H, J, K, L, N, O. Occasional in the Tennessee River; sporadic in the Ohio, upper Green, and Licking rivers. Known from one record each in the lower Cumberland River, Livingston/Marshall County (MSUMZ); Salt River, Spencer County (KNP); and Kentucky River, Henry/Owen counties (Tolin and King, 1986).

Truncilla truncata Rafinesque, 1820. Deertoe. A, B, E, F, G, H, J, K, L, N, O, P, Q, S. Occasional in the Ohio, Tennessee (below Kentucky Dam), Salt, and Licking rivers. Sporadic in the Green and Kentucky rivers and Tygarts Creek. Rare in the Mississippi River, Hickman County (KNP); upper Cumberland River (below the Falls), Rockcastle County (KNP); and Kinniconick Creek, Lewis County (C. R. Burchett, Jr., pers. comm.).

Uniomerus tetralasmus (Say, 1831). Pondhorn. B, C, E, F, G, H, K. Rare and known only from one or two collections each in the Tennessee River, McCracken County (KNP); lower

Cumberland River (Barkley Reservoir), Trigg County (MSUMZ); Tradewater River, Webster County (Warren and Call, 1983); and lower Green River, Daviess (Warren and Call, 1983) and Ohio (MSUMZ) counties.

Villosa fabalis (Lea, 1831). Rayed bean. B, J, K, N, O. Known historically from the Ohio River, Kenton County (Lea, 1870); upper Green River, Grayson (KNP), Green (HDA), Hart (Stansbery, 1965; KNP), and Warren (ANSP; UMMZ) counties; Salt River, Spencer County (KNP); Kentucky River, Gallatin and Scott counties (KNP); and Licking River, Pendleton County (KNP). The most recent collection of live or fresh-dead specimens is from the upper Green River (Stansbery, 1965). E/2.

Villosa iris (Lea, 1829). Rainbow. B, F, H, J, K, L, N, O, P, Q. Generally distributed in the upper Cumberland River (below the Falls); occasional in Kinniconick Creek; and sporadic in the lower Cumberland, upper Green, Kentucky, and Licking rivers and Tygarts Creek.

Villosa lienosa (Conrad, 1834). Little spectaclecase. B, E, F, H, J, K, L, N, O, P, Q, R, S. Occasional in Kinniconick Creek; sporadic in the upper Green, Salt, and Kentucky rivers. Known from single collections in the Tennessee River, Graves County (INHS); Licking River, Bath County (KNP); Little Sandy River, Carter County (KNP); and Big Sandy River, Lawrence County (Tolin and Schettig, 1984). S/-.

Villosa ortmanni (Walker, 1925). Kentucky creekshell. H, J. Endemic to and occasional in the upper Green River, where it persists in the Barren, Green, and Nolin rivers. E/2.

Villosa taeniata (Conrad, 1834). Painted creekshell. Cumberlandian species. F, L. Occasional in the upper Cumberland River (below the Falls); sporadic in the lower Cumberland River.

Villosa trabalis (Conrad, 1834). Cumberland bean. Cumberlandian species. L. Occasional and rare in the upper Cumberland River (below the Falls). Persists in Buck Creek, Pulaski County (Schuster *et al.*, 1989); Rockcastle River, Jackson, Laurel, Pulaski, and Rockcastle counties (Thompson, 1985; KNP; OSUMZ); and the Big and Little South Forks Cumberland River, McCreary and Wayne counties (Starnes and Bogan, 1982; KNP). E/E.

Villosa vanuxemensis vanuxemensis (Lea, 1838). Mountain creekshell. Cumberlandian species. F. Sporadic and rare in the lower Cumberland River. Specimens reported from the upper Cumberland River (below the Falls) by Neel and Allen (1964) are considered mis-identified *T. lividus* (Stansbery, 1976c; Starnes and Bogan, 1982). T/-.

DISCUSSION

The 103 unionid taxa reported herein for Kentucky (excluding the provisional record for *Q. sparsa*) compares favorably with the 104 taxa listed by Bickel (1967) and 103 species presented by Schuster (1988). Bickel's (1967) list can be reduced to 89 taxa, including two not recognized by us (*Actinonaias* [= *Venustaconcha*] *ellipsiformis* (Conrad, 1836) and *Pleurobema edgariana* [= *Fusconaia cor* (Conrad, 1834)]), by using Turgeon *et al.* (1988) as a nomenclatural standard.

Fifteen additional species reported here (*Alasmidonta atropurpurea*, *Anodonta suborbiculata*, *Epioblasma biemarginata*, *E. propinqua*, *E. sampsonii*, *E. stewardsoni*, *Glebula rotundata*, *Lasmigona subviridis*, *Lexingtonia dolabelloides*, *Obovaria retusa*, *Plectomerus dombeyanus*, *Potamilus purpuratus*, *Quadrula apiculata*, *Q. tuberosa*, and *Toxolasma texasensis*) were discovered in Kentucky subsequent to Bickel (1967), mentioned in literature he did not review, or simply overlooked. The only qualitative differences between Schuster (1988), who also used the nomenclature of Turgeon *et al.* (1988), and our list are that he included *V. ellipsiformis* as a dubious member of the fauna, and we added *Q. tuberosa*. *Glebula rotundata* is a Gulf Coastal species generally found within about 200 km of the Gulf (Gordon, 1983) and is probably not a permanent member of the fauna.

Faunal richness varies considerably between and within drainage systems (Tables 3 and 4). For example, only a single species (*Anodonta grandis*) is known from Terrapin Creek (region D) whereas at least 80 are known from the Ohio River. Eighty-seven taxa are known from the Cumberland River of Kentucky but only 11 of these have been documented above Cumberland Falls, a major barrier to dispersal. Contrary to previous reports that the mainstream Mississippi River downstream from St. Louis is either devoid of unionids or supports few species because of the Missouri River silt or mud load (Bartsch, 1916; van der Schalie and van der Schalie, 1950; Oesch, 1984), 22 taxa have been found in the river adjacent Kentucky, including 19 from one bed in Hickman County.

Kentucky's rich fauna is the result of the presence of three unionid assemblages (Ortmann, 1924, 1926; Clench and van der Schalie, 1944). The unique Cumberlandian fauna is restricted to the Cumberland and Tennessee river basins from their headwaters downstream to Clarksville, Tennessee and Muscle Shoals, Alabama, respectively (Ortmann, 1924, 1925). Twenty-one members of this fauna as defined by Ortmann (1924), excluding the problematic *Quadrula sparsa*, and refined by Johnson (1980) and Gordon and Layzer (1989) are known from the Cumberland River in Kentucky (regions F, L, and M). Ortmann (1924) also suggested that several other Cumberlandian unionids had invaded the Ohio River drainage but did not name them. Johnson (1980) identified this group as including *Epioblasma flexuosa*, *E. personata*, *E. propinqua*, *E. sampsonii*, *Lampsilis abrupta*, *Plethobasus cicatricosus*, and *Toxolasma lividus*. Two Cumberlandian species, *Dromus dromas* and *E. arcaeiformis*, were recently discovered at archaeological sites in the the lower Cumberland and Tennessee river drainages (Casey, 1986) outside the Cumberlandian region and are probably also members of this group.

The Interior Basin or Mississippian fauna is broadly distributed throughout the Mississippi and Ohio drainages (van der Schalie and van der Schalie, 1950) and includes 73 taxa in Kentucky. Johnson (1980) subdivided this area to create the Ohioan Region which in addition to Mississippian species includes several (16) of Ohioan origin (e.g., *Epioblasma obliquata*, *Hemistena lata*, *Obovaria retusa*, and *Villosa fabalis*). He also mentioned the Gulf Coastal Region, which includes the Mississippi River drainage downstream from the Ohio River exclusive of the Ozarkian Region, but did not identify

Table 3. Unionid distribution by major river drainage (A, Mississippi River mainstream; B, Ohio River mainstream and minor tributaries; C, Mississippi River tributaries; D, Terrapin Creek and other small tributaries of Obion River; E, lower Tennessee and Clarks rivers; F, lower Cumberland River; G, Tradewater River; H, lower Green River; J, upper Green and Barren rivers; K, Salt River; L, upper Cumberland River below Cumberland Falls; M, upper Cumberland River above Cumberland Falls; N, Kentucky River; O, Licking River; P, Kinniconick and Salt Lick creeks; Q, Tygarts Creek; R, Little Sandy River; S, Big Sandy River) (* = included provisionally).

Taxa	A	B	C	D	E	F	G	H	J	K	L	M	N	O	P	Q	R	S
<i>Actinonaias</i>																		
<i>ligamentina</i>		X			X	X	X	X	X	X	X		X	X		X		X
<i>pectorosa</i>						X					X	X						
<i>Alasmidonta</i>																		
<i>atropurpurea</i>											X	X						
<i>marginata</i>		X				X			X	X	X	X	X	X			X	
<i>viridis</i>		X				X		X	X	X	X	X	X	X				
<i>Amblema</i>																		
<i>p. plicata</i>	X	X			X	X	X	X	X	X	X		X	X	X	X	X	X
<i>Anodonta</i>																		
<i>grandis</i>	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X
<i>imbecillis</i>	X	X	X		X	X	X	X	X	X	X		X	X				
<i>suborbiculata</i>		X	X		X	X		X	X									
<i>Anodontoides</i>																		
<i>ferussacianus</i>		X				X	X	X	X	X	X	X	X	X		X		
<i>Arcidens</i>																		
<i>confragosus</i>	X	X	X		X	X	X	X	X									
<i>Cumberlandia</i>																		
<i>monodonta</i>		X							X		X							
<i>Cyclonaias</i>																		
<i>tuberculata</i>		X			X	X		X	X	X	X		X	X				X
<i>Cyprogenia</i>																		
<i>stegaria</i>		X			X	X		X	X	X	X		X	X		X		X
<i>Dromus</i>																		
<i>dromas</i>					X	X					X							
<i>Ellipsaria</i>																		
<i>lineolata</i>		X			X	X		X	X		X		X	X				
<i>Elliptio</i>																		
<i>crassidens</i>	X	X			X	X	X	X	X	X	X		X	X	X	X	X	X
<i>dilatata</i>		X			X	X		X	X	X	X	X	X	X	X	X	X	
<i>Epioblasma</i>																		
<i>arcaeiformis</i>					X	X					X							
<i>biemarginata</i>											X							
<i>brevidens</i>											X							
<i>capsaeformis</i>											X							
<i>flexuosa</i>		X			X	X		X	X	X			X					
<i>f. florentina</i>											X							
<i>f. walkeri</i>											X							
<i>haysiana</i>											X							
<i>lewisii</i>		X									X							
<i>o. obliquata</i>		X						X	X		X		X	X				
<i>o. perobliqua</i>		X?																
<i>personata</i>		X																
<i>propinqua</i>		X																
<i>sampsonii</i>		X						X					X					
<i>stewardsoni</i>											X							
<i>t. rangiana</i>		X						X	X	X			X	X				
<i>t. torulosa</i>		X			X	X			X	X				X				
<i>triquetra</i>		X				X		X	X	X	X		X	X	X	X		

Table 3. Continued.

Taxa	A	B	C	D	E	F	G	H	J	K	L	M	N	O	P	Q	R	S
<i>Fusconaia</i>																		
<i>ebena</i>	X	X			X	X			X		X			X				
<i>flava</i>		X			X	X	X	X	X	X	X		X	X	X	X	X	X
<i>subrotunda</i>		X			X	X		X	X	X	X		X	X				X
<i>Glebula</i>																		
<i>rotundata</i>		X																
<i>Hemistena</i>																		
<i>lata</i>		X							X		X		X					
<i>Lampsilis</i>																		
<i>abrupta</i>		X			X	X			X	X	X							
<i>cardium</i>		X			X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>fasciola</i>		X				X			X	X	X	X	X	X	X	X		
<i>ovata</i>		X			X	X		X	X	X	X	X						
<i>siliquoidea</i>		X	X		X	X	X	X	X	X				X	X	X	X	X
<i>teres</i>	X	X	X		X	X	X	X	X	X				X	X			
<i>Lasmigona</i>																		
<i>c. complanata</i>	X	X	X		X	X	X	X	X	X			X	X			X	X
<i>compressa</i>		X														X	X	
<i>costata</i>		X				X	X	X	X	X	X		X	X	X	X	X	X
<i>subviridis</i>																X		
<i>Leptodea</i>																		
<i>fragilis</i>	X	X	X		X	X	X	X	X	X	X		X	X	X	X	X	X
<i>leptodon</i>		X							X		X		X					
<i>Lexingtonia</i>																		
<i>dolabelloides</i>						X												
<i>Liguma</i>																		
<i>recta</i>	X	X			X	X		X	X	X	X		X	X		X		X
<i>subrostrata</i>		X	X		X		X	X										
<i>Medionidus</i>																		
<i>conradicus</i>						X					X							
<i>Megaloniaias</i>																		
<i>nervosa</i>	X	X			X	X	X	X	X	X	X		X	X		X		X
<i>Obliquaria</i>																		
<i>reflexa</i>	X	X			X	X	X	X	X	X	X		X	X				
<i>Obovaria</i>																		
<i>olivaria</i>	X	X			X	X		X	X		X		X					
<i>retusa</i>		X			X	X		X	X		X		X					
<i>subrotunda</i>		X			X	X		X	X	X	X		X	X		X	X	X
<i>Pegias</i>																		
<i>fabula</i>						X					X							
<i>Plectomerus</i>																		
<i>dombeyanus</i>		X			X													
<i>Plethobasus</i>																		
<i>cicatricosus</i>		X				X												
<i>cooperianus</i>		X			X	X		X	X	X	X							
<i>cyphus</i>		X			X	X		X	X		X			X				
<i>Pleurobema</i>																		
<i>clava</i>		X			X	X			X	X			X	X				
<i>coccineum</i>		X			X	X		X	X	X	X		X	X		X		
<i>cordatum</i>		X			X	X		X	X	X	X		X	X		X		
<i>oviforme</i>						X					X							

Table 3. Continued.

Taxa	A	B	C	D	E	F	G	H	J	K	L	M	N	O	P	Q	R	S
<i>plenum</i>		X						X	X		X		X	X				
<i>pyramidatum</i>		X			X	X		X	X	X	X		X	X				
<i>Potamilus</i>																		
<i>alatus</i>	X	X	X		X	X	X	X	X	X	X		X	X	X	X	X	X
<i>capax</i>		X				X		X										
<i>ohiensis</i>	X	X	X		X	X		X	X	X	X		X	X				X
<i>purpuratus</i>	X	X																
<i>Ptychobranchus</i>																		
<i>fasciolaris</i>		X			X	X		X	X	X	X		X	X	X	X	X	X
<i>subtentum</i>						X					X							
<i>Quadrula</i>																		
<i>apiculata</i>	X	X			X			X	X									
<i>c. cylindrica</i>		X			X	X		X	X	X	X		X	X				X
<i>fragosa</i>		X				X												
<i>metanevra</i>		X			X	X		X	X		X		X	X				
<i>nodulata</i>	X	X			X	X	X	X		X			X	X				
<i>p. pustulosa</i>	X	X	X		X	X	X	X	X	X	X		X	X	X	X	X	X
<i>quadrula</i>	X	X	X		X	X	X	X	X	X	X		X	X		X	X	X
<i>sparsa</i>											X*							
<i>tuberosa</i>											X							
<i>Simpsonaias</i>																		
<i>ambigua</i>		X							X	X			X	X	X	X	X	
<i>Strophitus</i>																		
<i>undulatus</i>		X				X		X	X	X	X	X	X	X	X	X	X	X
<i>Toxolasma</i>																		
<i>lividus</i>		X				X		X	X	X	X							
<i>parvus</i>		X	X		X			X	X	X	X	X	X	X			X	
<i>texasensis</i>					X		X											
<i>Tritogonia</i>																		
<i>verrucosa</i>	X	X	X		X	X	X	X	X	X	X		X	X	X	X	X	X
<i>Truncilla</i>																		
<i>donaciformis</i>		X			X	X	X	X	X	X	X		X	X				
<i>truncata</i>	X	X			X	X	X	X	X	X	X		X	X	X	X		X
<i>Uniomerus</i>																		
<i>tetralasmus</i>		X	X		X	X	X	X		X								
<i>Villosa</i>																		
<i>fabalis</i>		X							X	X			X	X				
<i>iris</i>		X				X		X	X	X	X		X	X	X	X		
<i>lienosa</i>		X			X	X		X	X	X	X		X	X	X	X	X	X
<i>ortmanni</i>								X	X									
<i>taeniata</i>						X					X							
<i>trabalis</i>											X							
<i>v. vanuxemensis</i>						X												
TOTAL TAXA	22	80	16	1	55	70	26	60	66	53	72	11	56	53	20	31	22	26

characteristic elements of the fauna. Finally, *Lasmigona subviridis* is known from Tygarts Creek and is considered an Atlantic Slope species (Johnson, 1980) that could have originated in the western Alleghenies (Ortmann, 1913).

Additions to the fauna can be expected as a result of taxonomic revisions (e.g. the *Villosa iris* and *Pleurobema*

oviforme complexes) and the discovery of Interior Basin species that occur in adjacent states, especially those in the Gulf Coastal Plain. Figures 2 and 3 suggest that the lower Green, Salt, and mainstream Kentucky rivers and streams on the Cumberland Plateau have not been adequately sampled, but most drainages can yield important finds. For example,

recent discoveries include *Alasmidonta atropurpurea* in the upper Cumberland River above and below the Falls (Call and Parmalee, 1981) and *Potamilus capax* from the lower Cumberland River (Sickel, 1987a). Small streams and difficult to sample deep, flowing water habitats in medium to large rivers are perhaps most worthy of further sampling.

During the last 200 years, human activities have greatly altered Kentucky's waters and landscape and severely affected unionid populations. These impacts were reviewed by Fuller (1974) and Havlik and Marking (1987) and include altered physicochemical conditions, channelization, dams, commercial unionid fishing, mining wastes, pesticides, and others. As a result of such alterations in Kentucky, 18 taxa (excluding *Quadrula sparsa*), 17% of the fauna, are considered extinct or have probably been extirpated from the state. Of the 84 extant taxa, 34 (40%) have been assigned federal or state conservation statuses in recognition of their rarity (Warren *et al.*, 1986). Unionid diversity probably has been reduced in all drainages. For example, the Ohio River historically supported at least 80 taxa and was among the most diverse rivers in the nation. But as a result of massive alterations associated with navigation improvements that began in 1830 (United States Army Corps of Engineers, 1981), industrial and agricultural development, and human population expansion, only 45 taxa (56%) are known to persist, several only as remnant populations or individuals. Of the 72 taxa that inhabited the upper Cumberland River below the Falls, half are extinct, extirpated from the state, or no longer occur in this region (L), and 11 of the 36 that persist are rare at the state or federal level. These changes in faunal composition are primarily the result of impacts from the closure and operation of Wolf Creek Dam and coal mining. Miller *et al.* (1984) reported that with the exception of single specimens of *Cumberlandia mono-*

donta and *Cyclonaias tuberculata* the rich fauna documented from the upper Cumberland River below the Falls by Wilson and Clark (1914) and Neel and Allen (1964) has been extirpated by physical and chemical changes from the dam operation. As indicated by archaeological site records (Parmalee, 1960; Call and Robinson, 1983; Casey, 1986; OSUMZ), prehistoric unionid distributions could have differed significantly from historic patterns (e.g. *Dromus dromas*, *Epioblasma arcaeformis*, *E. flexuosa*, *E. sampsonii*).

Habitat alteration has affected faunal and taxonomic groups differentially. With the exception of *Actinonaias pec-torosa*, *Medionidus conradicus*, and *Villosa taeniata*, the remaining 18 Cumberlandian species comprise 35% of the rare, extirpated, or extinct unionids of Kentucky. Thirteen of the 18 members of the genus *Epioblasma* are considered extirpated or extinct, and the remainder persist precariously. These unionids typically inhabited medium to large rivers with swift current (Gordon and Layzer, 1989), habitats that have been all but lost in Kentucky.

Kentucky's unionid fauna is perhaps the most endangered group of organisms in the state. A large number of taxa are on the verge of extirpation from the state or extinction (e.g. *Alasmidonta atropurpurea*, *Cumberlandia monodonta*, *Epioblasma brevidens*, *E. capsaeformis*, *E. torulosa rangiana*, *Lampsilis abrupta*, *Obovaria retusa*, *Plethobasus cooperianus*, *Pleurobema clava*, *P. pyramidatum*, *Quadrula fragosa*, *Toxolasma lividus*). Unless efforts are made soon to cryopreserve gametes/zygotes or secure and maintain representatives of these taxa in controlled laboratory environments pending application of culture techniques, these organisms will be lost as part of our natural heritage. Equally important is the protection of streams that support rare unionids or diverse faunas, such as Buck Creek, Big South Fork Cumberland River, segments of the Green River, Horse Lick Creek, and the Tennessee River downstream from Kentucky Dam. Although once quite common in Kentucky, the natural habitats of unionids as well other aquatic organisms have been greatly altered or lost. Those remaining constitute important refugia for the fauna and important epicenters for reinvasion.

ACKNOWLEDGMENTS

The following individuals and/or agencies generously shared information or provided access to collections in their care: H. D. Athearn; D. L. Batch and J. C. Williams, ECU; R. T. Bay, R. G. Biggins, D. B. Winford, and J. D. Williams, USFWS; A. E. Bogan, ANSP; K. Boss, MCZ; C. R. Burchett, Jr.; J. B. Burch and D. J. Eernisse, UMMZ; B. M. Burr, Southern Illinois University at Carbondale; R. S. Butler, Florida Game and Freshwater Fish Commission; S. M. Call, S. Evans, and R. Houpp, Kentucky Division of Water; K. S. Cummings and C. A. Mayer, INHS; G. J. Fallo; C. H. Gooch, W. J. Pardue, and D. C. Wade, Tennessee Valley Authority; D. T. Henley, L. E. Kornman, K. W. Prather, D. E. Stephens, and A. F. Surmont, Jr., Kentucky Department of Fish and Wildlife Resources; R. Hershler and P. R. Greenhall, NMNH; B. R. Kuhajda, University of Alabama; J. R. MacGregor, United States Forest Service; S. P. Rice, Kentucky Department of Transportation; J. E. Rawlins, CM; J. B. Sickel, MSUMZ; A. Solem, FMNH; D. H. Stansbery, K. G. Borror, W. R. Haag, and G. T. Watters, OSUMZ;

Table 4. Unionid diversity by major river drainage in Kentucky (letters refer to figure 1).

River Drainage	Number of Species
Mississippi (A)	22
Ohio (B)	80
Mississippi tributaries (C)	16
Terrapin Creek (D)	1
Lower Tennessee (E)	55
Lower Cumberland (F)	70
Upper Cumberland below Falls (L)	72
Upper Cumberland above Falls (M)	11
Cumberland (entire drainage)	87
Tradewater (G)	26
Lower Green (H)	60
Upper Green (J)	66
Green (entire drainage)	71
Salt (K)	53
Kentucky (N)	56
Licking (O)	53
Kinniconick and Salt Lick creeks (P)	20
Tygarts Creek (Q)	31
Little Sandy (R)	22
Big Sandy (S)	26

and R. W. Taylor, MU.

Several individuals graciously provided logistic, field, or other support including R. G. Biggins; R. S. Butler; K. S. Cummings; L. B. Starnes, USFWS; W. C. Starnes, NMNH; and T. J. Timmons, MSUMZ. D. H. Stansbery graciously identified or confirmed the identification of numerous specimens. S. M. Call and J. B. Sickel reviewed the manuscript.

The following former and present KNP staff members provided field assistance and other support: J. D. Bender, C. T. Bloom, S. M. Call, K. E. Camburn, R. J. DiStefano, M. Evans, G. J. Fallo, W. R. Haag, D. H. Harker, Jr., R. R. Hannan, W. C. Houtcooper, B. Palmer-Ball, Jr., R. Rhew, and D. E. vanNorman. Special thanks to R. R. Hannan, Director (KNP), for providing the opportunity to undertake this project.

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Date of manuscript acceptance: 4 December 1990

OZARKIAN FRESH-WATER MUSSELS (UNIONOIDEA) IN THE UPPER ELEVEN POINT RIVER, MISSOURI

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ABSTRACT

Fourteen species of fresh-water mussels (Bivalvia: Unionoidea) were collected in 1990 from the upper reaches of the Eleven Point River in Oregon County, Missouri. The collections constitute range extensions for all 14 species, including five species newly recorded for the Eleven Point River in Missouri and nine upstream records for species previously documented in the Missouri section of the river. The collections are dominated by four endemic Ozarkian species [*Fusconaia ozarkensis* (Call), *Lampsilis reeviana brevicula* (Call), *Venustaconcha pleasi* (Marsh), and *Ptychobranthus occidentalis* (Conrad)], two of which are rare/uncommon in Missouri. There is significant variation in species composition along the 26 km section of river studied, which could be related to the inflow of numerous springs and a considerable downstream increase in stream discharge. The upper Eleven Point River may be a suitable refuge for the conservation of Ozarkian mussel communities.

The Interior Highlands region of southern Missouri, northern Arkansas, and eastern Oklahoma is an isolated upland comprised of two rugged physiographic provinces, the Ozark Plateau and the Ouachita Mountains. Streams draining this area are inhabited by many species of aquatic organisms that live nowhere else. The list of endemic taxa includes at least 14 species of fish (Pflieger, 1975), seven gastropods (Gordon, 1980), and nine species of fresh-water mussels (cf. Gordon, 1980; Johnson, 1980; Oesch, 1984).

Many mussel species endemic to the Interior Highlands are adapted to the clear, high-gradient streams with coarse bed material that are typical of the region's drainages (see Oesch, 1984; Warren, 1991). Because of their rather limited habitat tolerances, these mussels can be highly susceptible to the effects of recent ecological changes stemming from pollution, excess siltation, and the damming of streams. The threat to these species is underscored by the fact that there are clear parallels between the Ozarkian faunal province of the Interior Highlands and the Cumberlandian faunal province of the Cumberland Plateau region of eastern Kentucky and Tennessee (van der Schalie and van der Schalie, 1950). Several of the endemic Ozarkian mussel species have closely related counterparts in the Cumberland region. These pairs of species share not only a common ancestry [e.g. *Fusconaia ozarkensis* (Call) and *F. barnesiana* (Lea); see Johnson, 1980; Ortmann, 1917], but also a preference for clear, energetic streams with coarse bed materials. This parallel is important because the Cumber-

landian mussel fauna has been depleted or destroyed in many areas due to the effects of modern development (Bogan and Parmalee, 1983; Isom, 1969; Stansbery, 1970, 1971). The Ozarkian fauna is susceptible to the same fate, and a majority of its endemic taxa have already appeared on lists of rare and endangered species (see Harris and Gordon, 1987; Nordstrom *et al.*, 1977; Oesch, 1984).

To expand on a recent statement by Harris and Gordon (1987:54), there is an urgent need to better delineate the distribution and biology of fresh-water mussels throughout the Interior Highlands region, especially for endemic Ozarkian taxa with restricted environmental tolerances. This paper reports a preliminary survey of Unionacea in the upper Eleven Point River (White River Basin) in Oregon County, Missouri. Previous work in the White River Basin includes incidental remarks by Call (1895) and Utterback (1915-1916) in their statewide synopses of the mussels of Arkansas and Missouri, respectively. Zoogeographic studies include general surveys by Utterback (1917), Johnson (1980), and Gordon *et al.* (1980), which provide lists of taxa at the scale of the drainage basin. Oesch (1984) maps specific data on species locations for the central section of the Eleven Point River in Missouri, but his coverage does not extend into the upper reaches of the river.

The main objectives of the present study are to (1) document extant populations of mussel species in a 26 km stretch of the upper Eleven Point River, and (2) describe the composition and geographical variation of mussel communities in this area. The data have important implications

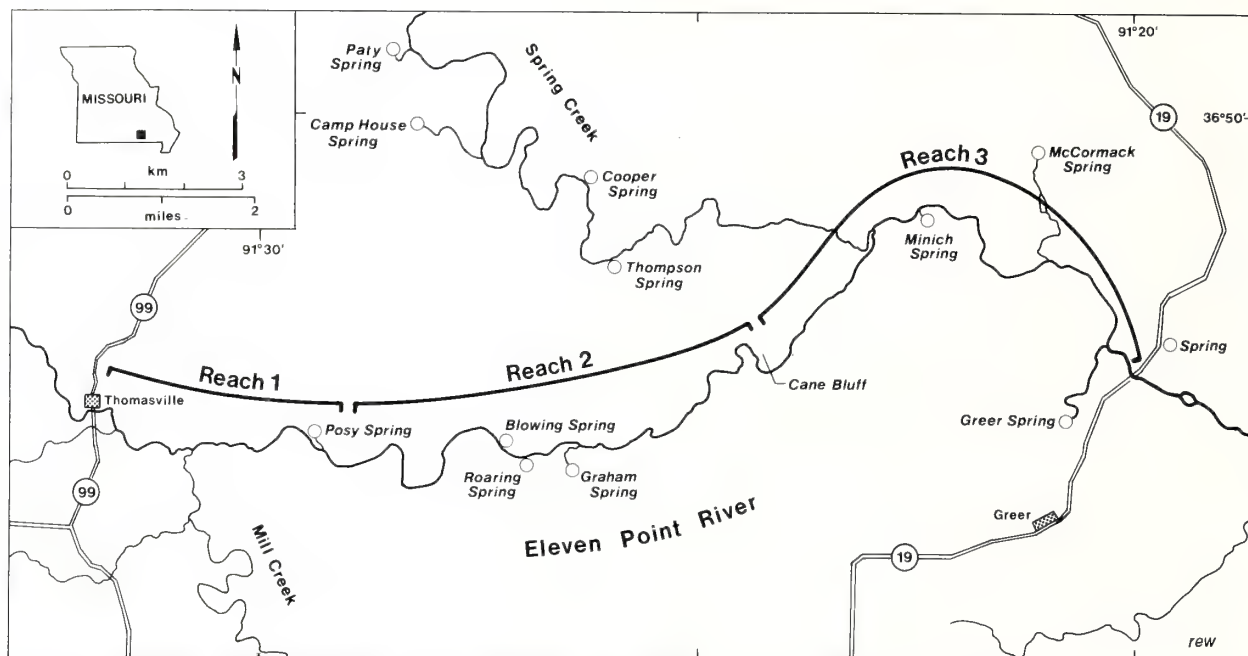


Fig. 1. Map of collection areas (Reaches 1-3) along the upper Eleven Point River in Oregon County, Missouri.

for the conservation of rare and potentially endangered Ozarkian species.

MATERIALS AND METHODS

The Eleven Point River is a clear, spring-fed Ozark stream. Its headwaters are in Howell County, Missouri, near the town of Willow Springs. The river flows southeastward across the Salem Plateau for about 241 km before joining the Spring River, a tributary of the Black River (White River Basin), in Randolph County, Arkansas (see Johnson and Beadles, 1977). The Eleven Point River flows through a steeply dissected karst landscape. Its flood plain is often narrow, and bluffs of Ordovician dolomite and sandstone tower up to 129 m above the valley floor (see Bretz, 1965).

The Eleven Point Basin is mainly an agricultural area today, with pastures on the high plateaus and low flood plains, and oak-hickory forest on the intervening slopes. Farms were established along the river as early as 1819, but population density remained low until well after the Civil War (Sauer, 1920). Logging became an important industry during the last quarter of the 19th century, reaching its peak about 1900 and declining thereafter. Deforestation probably increased rates of hillslope erosion in the area, but its historical impact on the water quality and geomorphology of the Eleven Point River is unknown.

The study area is a 26.4 km stretch of the Eleven Point River that runs downstream from Thomasville (State Highway 99) to the State Highway 19 bridge northeast of Greer (Fig. 1). This area comprises the upper reaches of the Eleven Point National Wild and Scenic River, which was established in 1968 and is maintained by the Mark Twain National Forest. Stream

elevation drops from about 189.6 to 158.2 m AMSL in this area, and its gradient is relatively steep (1.2 m/km). The stream channel consists of a series of shallow riffles, raceways, and deeper pools. Its current velocity varies from slow to swift, and its substrate consists primarily of cobbles, gravel, and some sand.

Seven major springs enter the river in the study area, increasing its discharge considerably between Thomasville and the Highway 19 bridge. Greer Spring, the second largest spring in Missouri (Vineyard and Feder, 1974), enters the Eleven Point River 0.6 km upstream from the Highway 19 bridge, where it reportedly triples the discharge of the stream. The spring had an average discharge of 9.55 m³/sec during its 68-year period of record (1922-1988; data from U. S. Geological Survey, Rolla). Discharge data are not available for the Eleven Point River above Greer Spring, although at Bardley, Missouri, 26 km downstream from Greer, its discharge averaged 21.66 m³/sec during the 1922-1988 period of record (data from U. S. Geological Survey, Rolla).

The downstream increase in discharge has a perceptible effect on the river channel (Fig. 2), and could in turn be related to variation in mussel habitat. Between the upper and lower ends of the study area, channel width increases from about 10 to 22 m, channel depth increases from about 45 to >93 cm, and current velocity increases from moderate or swift to very swift. (Channel dimensions were measured at raceways.) There could also be a downstream change in substratum composition, but this has not been measured.

All mussels and mussel shells were collected by hand from gravel bars, riffles, and shallow areas along deeper raceways and pools (9-10 June 1990). The collection is a "grab" sample that was not obtained using quantitative sampl-



Fig. 2. Two views of the Eleven Point River, Oregon County, Missouri, showing (a) the relatively small channel at the upper end of Reach 1, looking downstream from below the Highway 99 bridge near Thomasville, and (b) the larger spring-fed channel at the lower end of Reach 3, looking downstream from below the Highway 19 bridge near Greer (February, 1991).

ing techniques (cf. Miller and Payne, 1988). The sample is presumably representative of the relative abundance of various species, but it is not appropriate for estimating the size, density, or demographic characteristics of local mussel populations.

Most of the specimens collected were dead. Some could have been residues of muskrat predation, but many appear to have died when they were dislodged by flood waters and displaced outside of the river channel, where they perished after the river returned to its banks. Water levels in the river were about 23-25 cm higher than normal at the time of our survey because of unusually heavy spring rainfall, but the water levels were receding.

Live individuals were collected as one sample and stored on ice. Dead individuals were collected from three separate reaches of the river (Fig. 1), cleaned, and stored dry. Reach 1, the farthest upstream, is 5.6 km long and runs from

the Thomasville access to a point below Posy Spring (0-5.6 km below Thomasville). Reach 2 is 9.4 km long and runs from the Posy Spring locality to the Cane Bluff access (5.6-15.0 km below Thomasville). Reach 3 runs 11.4 km from Cane Bluff to the Highway 19 bridge (15.0-26.4 km below Thomasville).

Dead shells were classified by valve condition. 'Fresh shells' are specimens with the nacre and periostracum intact and well-preserved. This category includes all paired valves that were still joined at the dorsal ligament. 'Eroded shells' have weathered, worn, or poorly preserved nacre and periostracum. Some broken shells were included in this category, but all retain the dorsal hinge line and are at least half complete.

Specimens were identified using a comparative collection at the Illinois State Museum, Springfield. Nomenclature follows Turgeon *et al.* (1988). All specimens are being curated at the Illinois State Museum.

RESULTS

Our collections in the upper Eleven Point River produced a total of 14 species of Unionacea (Table 1). All are represented by live or fresh-dead individuals. Nine species are upstream records for taxa previously documented by Oesch (1984) at or below the Highway 19 bridge in Missouri, including *Elliptio dilatata* (Rafinesque), *Fusconaia ozarkensis*, *Pleurobema coccineum* (Conrad), *Lampsilis cardium*

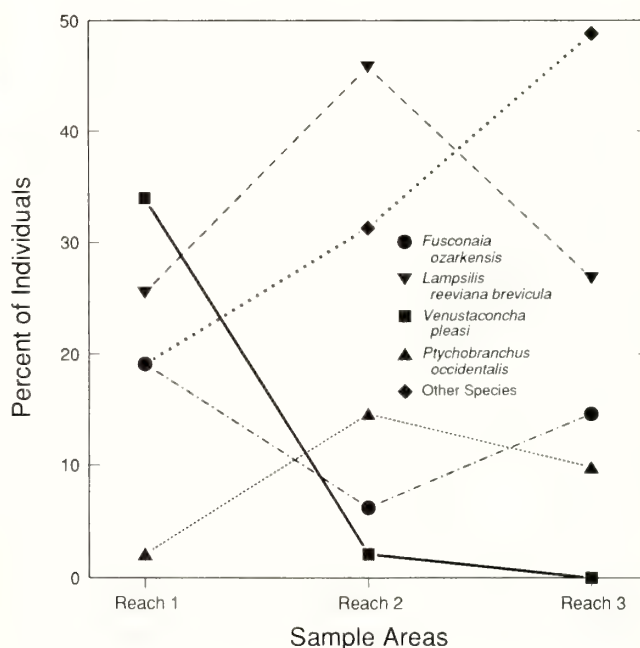


Fig. 3. Variation in the species composition of fresh-water mussels in three reaches of the upper Eleven Point River, Missouri. The four Ozarkian species in the collection (*Fusconaia ozarkensis*, *Lampsilis reeviana brevicula*, *Venustaconcha pleasi*, and *Ptychobranchus occidentalis*) are plotted individually. All 10 of the other taxa in the collection are Mississippian species.

Table 1. Fresh-water mussels from the Upper Eleven Point River, Oregon County, Missouri.

Taxon ¹	Live mussels	Dead mussels									Total	Percent Total
		Reach 1			Reach 2			Reach 3				
		Fresh	Eroded	Total	Fresh	Eroded	Total	Fresh	Eroded	Total		
<i>Anodonta imbecillis</i> Say, 1829	0	0	0	0	1	0	1	0	0	0	1	.6
<i>Lasmigona costata</i> (Rafinesque, 1820)	1	0	0	0	2	0	2	4	1	5	8	5.2
<i>Strophitus undulatus</i> (Say, 1817)	1	2	0	2	0	0	0	0	0	0	3	1.9
<i>Elliptio dilatata</i> (Rafinesque, 1820)	0	0	0	0	0	0	0	4	1	5	5	3.2
<i>Fusconaia flava</i> (Rafinesque, 1820)	0	0	0	0	2	0	2	0	0	0	2	1.3
<i>F. ozarkensis</i> (Call, 1887)	5	8	1	9	2	1	3	5	1	6	23	14.8
<i>Pleurobema coccineum</i> (Conrad, 1834)	1	0	0	0	0	1	1	3	2	5	7	4.5
<i>Lampsilis cardium</i> (Rafinesque, 1820)	0	2	0	2	5	2	7	1	1	2	11	7.1
<i>L. reeviana brevicula</i> (Call, 1887)	4	9	3	12	19	3	22	9	2	11	49	31.6
<i>L. siliquioidea</i> (Barnes, 1823)	0	0	0	0	1	1	2	1	1	2	4	2.6
<i>Venustaconcha pleasi</i> (Marsh, 1891)	5	15	1	16	1	0	1	0	0	0	22	14.2
<i>Villosa iris</i> (Lea, 1829)	0	2	1	3	0	0	0	1	0	1	4	2.6
<i>V. lienosa</i> (Conrad, 1834)	0	2	0	2	0	0	0	0	0	0	2	1.3
<i>Ptychobranchus occidentalis</i> (Conrad, 1836)	2	0	1	1	5	2	7	4	0	4	14	9.0
Number of individuals	19	40	7	47	38	10	48	32	9	41	155	99.9
Number of taxa	7	7	5	8	9	6	10	9	7	9	14	—

¹Nomenclature follows Turgeon *et al.* (1988). Taxonomic ordering is phylogenetic above the genus level (after Davis and Fuller 1981:246).

(Rafinesque), *L. reeviana brevicula* (Call), *Venustaconcha pleasi* (Marsh), *Villosa iris* (Lea), *V. lienosa* (Conrad), and *Ptychobranchus occidentalis* (Conrad). Johnson (1980:133) lists an additional location for *L. r. brevicula* (as *V. reeviana*) at Riverton. Of the live individuals in our collection, two *F. ozarkensis* and three *V. pleasi* were gravid females.

Only two species documented by Oesch (1984) in the Eleven Point River are missing from our collections: *Cyclonaias tuberculata* (Rafinesque) and *Ligumia recta* (Lamarck). Oesch found *C. tuberculata* near the Highway 19 bridge, and recorded both species downstream near the Missouri-Arkansas border at Billmore, Missouri (Oesch 1984:117, 197). Their absence in our collections could be due to sampling error, limited zoogeographic ranges, or both.

The five remaining taxa are new records for the Eleven Point River in Missouri. These species include *Anodonta imbecillis* Say, *Lasmigona costata* (Rafinesque), *Strophitus undulatus* (Say), *Fusconaia flava* (Rafinesque), and *Lampsilis siliquioidea* (Barnes). All of these species have been documented elsewhere in the Black River Basin of southern Missouri and

northern Arkansas (Gordon *et al.*, 1984; Oesch, 1984; Utterback, 1917), and it was not surprising to find them in the upper Eleven Point River. *Corbicula fluminea* (Müller), the intrusive Asian clam, was noted in all three reaches but was not collected.

There is a significant correlation between the species counts of live and dead individuals in the collection (Pearson's $r = .73$; $df = 12$; $p < .01$; see Rock, 1988:168). This test indicates that the two samples are proportionately representative of the same population. Nevertheless, large quantitative collections from stratified sample areas would undoubtedly provide more reliable data on the species composition of extant communities.

Judging from the relative frequencies of individuals in the collection, the mussel community in the upper Eleven Point River is dominated by four species. All are endemic Ozarkian taxa (Gordon, 1980; Johnson, 1980), and together they comprise 69.6% of the collection. In decreasing order of abundance, these are *Lampsilis r. brevicula* (31.6%), *Fusconaia ozarkensis* (14.8%), *Venustaconcha pleasi* (14.2%),

Table 2. Geographical variation in the species composition and diversity of fresh-water mussel communities along the Upper Eleven Point River in Oregon County, Missouri.

Variable	Reach 1	Reach 2	Reach 3
Leading dominant	<i>Venustaconcha pleasi</i>	<i>Lampsilis reeviana brevicula</i>	<i>Lampsilis reeviana brevicula</i>
Species (S)	8	10	9
Individuals (N)	47	48	41
Percent Ozarkian species (%S) ¹	50.0	40.0	33.3
Percent Ozarkian individuals (%N) ¹	80.9	68.7	51.2
Simpson index of species dominance (D)	.21	.25	.13
Shannon index of species richness (H')	1.69	1.73	2.02

¹ Ozarkian species represented in the collection include *Fusconaia ozarkensis*, *Lampsilis reeviana brevicula*, *Venustaconcha pleasi*, and *Ptychobranhus occidentalis* (cf. Gordon 1980; Johnson 1980).

and *Ptychobranhus occidentalis* (9.0%). The remaining species are members of the Mississippian faunal province and have extensive ranges in the Mississippi River basin (Johnson, 1980; Oesch, 1984).

There is significant geographical variation in the species composition of dead shells collected from the three sample reaches (Tables 1-2; Fig. 3). The three leading dominants in Reach 1, the upstream section, are *Venustaconcha pleasi* (34.0%), *Lampsilis r. brevicula* (25.5%), and *Fusconaia ozarkensis* (19.1%). In Reach 2 there is a substantial drop in the abundance of *V. pleasi* (2.1%). The leading dominant in Reach 2 is *L. r. brevicula* (45.8%), followed by *Ptychobranhus occidentalis* (14.6%) and *L. cardium* (14.6%). *L. r. brevicula* is also the leading dominant in Reach 3, the downstream section, although its relative abundance drops to only 26.8%. The second leading dominant in Reach 3 is *F. ozarkensis* (14.6%), followed by *L. costata*, *Elliptio dilatata*, and *P. coccineum* at 12.2% each.

Geographical variation in species composition is accompanied by substantial downstream decreases in the relative abundance of Ozarkian elements and corresponding increases in Mississippian elements (Table 2; Fig. 3). Ozarkian taxa comprise 50% of the species documented in Reach 1, 40% in Reach 2, and 33% of the species in Reach 3. There is a parallel decline in the relative abundance of Ozarkian individuals, although the curve is even steeper. Ozarkian species account for more than 80% of individuals in Reach 1, but only about 69% in Reach 2 and 51% in Reach 3. These trends could reflect either a downstream decrease in the density of Ozarkian species, an upstream decrease in the density of Mississippian taxa, or both.

There also appears to be geographical variation in species diversity (Table 2). Although the numbers of species (S) and individuals (N) are fairly constant among reaches, there are discernible patterns in indexes of species dominance and species richness. The Simpson index (D; Simpson,

1949)—a measure of dominance concentration that correlates inversely with the evenness of species proportions—peaks in Reach 2 and is lowest in Reach 1. This pattern reflects the unusually high relative abundance of *Lampsilis r. brevicula* in Reach 2 and the relatively uniform proportions of various taxa in Reach 3. The Shannon index (H'; Shannon and Weaver, 1949)—a measure of species richness (see Magurran, 1988)—increases monotonically downstream from Reach 1 to Reach 3. This trend indicates that species diversity is relatively low in upstream areas dominated by Ozarkian taxa, but increases downstream where Mississippian taxa are relatively abundant. It should be cautioned that these patterns are based on rather limited data, and additional fieldwork is needed to confirm their validity.

DISCUSSION AND CONCLUSIONS

In a discussion of the prospects of rare and endangered fresh-water mussels in Arkansas, Harris and Gordon (1987) suggest that certain sections of the Eleven Point River and several other streams may offer suitable habitat for sustaining endangered species. However, in an assessment of the remaining habitat available to *Venustaconcha pleasi*, Oesch (1984:153) remarks that most of the Eleven Point River offers only "marginal habitat" because of its many tributary springs. Oesch (1984:229) suggests elsewhere that water from large springs is too cold and nutrient-poor for the survival of most mussel species. In a similar vein, Buchanan (1984:85) states that cold springs tend to reduce the abundance and species diversity of mussels in the Current River basin, the next drainage east of the Eleven Point Basin.

Despite these suggestions, our collections indicate there is a significant community of mussels in the upper reaches of the Eleven Point River, where spring water accounts for much of the river's discharge. It is true that the total number of known species (14) is low in comparison with the

Spring River, Black River, and other large streams in the White River Basin (see Gordon, 1982; Gordon *et al.*, 1984; Utterback, 1917). However, the Eleven Point River apparently provides suitable habitat for four endemic Ozarkian species (*Fusconaia ozarkensis*, *Lampsilis r. brevicula*, *Venustaconcha pleasi*, *Ptychobranhus occidentalis*), which together comprise almost 70% of the individuals collected. Moreover, three of these species have appeared on state or national lists of rare and endangered fresh-water mussels (*F. ozarkensis* [Nordstrom *et al.*, 1977]; *V. pleasi* [Nordstrom *et al.*, 1977]; *P. occidentalis* [Stansbery, 1971]). None is currently on federal or state lists of protected species (Fish and Wildlife Service, 1989; Missouri Department of Conservation, 1986), but *F. ozarkensis* and *V. pleasi* are rare/uncommon in Missouri (Dennis Figg, pers. comm., 1990).

There is significant downstream variation in species composition in the study area, which could be related to the inflow of numerous springs and a considerable increase in stream discharge. The abundance of *Venustaconcha pleasi* in the upstream sample area (Reach 1) suggests it is primarily a headwater species that could be most common in the upper reaches of Ozark streams (Fig. 3). Spatial patterning in the relative abundance of *V. pleasi* has not been documented elsewhere, but our observations are consistent with Gordon's (1982) qualitative study of the White River in Arkansas, where *V. pleasi* is restricted to the upper reaches of the stream above Fayetteville. *Lampsilis r. brevicula* is the most abundant species in the middle and lower sample areas (Reaches 2-3), where most of the tributary springs enter the river. This correlation is consistent with Oesch's (1984:229) suggestion that the various subspecies of *L. reeviana* are unusually tolerant of streams below the outlets of major springs. *Fusconaia ozarkensis* has a bimodal pattern of abundance, with peaks in the upper and lower sample areas (Reaches 1 & 3), while *Ptychobranhus occidentalis* parallels the distribution of *L. r. brevicula* with a peak in the middle sample area (Reach 2). Considering the rather small samples of *F. ozarkensis* and *P. occidentalis*, the significance of their compositional patterns is questionable. Other taxa, all of which are Mississippian species, tend to increase monotonically downstream.

There is a downstream increase in species diversity in the study area that correlates inversely with downstream decreases in the qualitative and apparent quantitative abundance of Ozarkian taxa and individuals. The Ozarkian decline could reflect increased competition with Mississippian faunal elements in the lower reaches of the river, although quantitative collections are needed to test this possibility.

The upper Eleven Point River in Oregon County, Missouri, is a potential refuge for the conservation of Ozarkian mussel communities. Further research is needed to measure the size, density, and demographic structure of mussel populations in this area, as well as to assess their habitat tolerances and reproductive systems in greater detail. The section of river discussed here is currently being protected in accordance with the National Wild and Scenic Rivers Act, and it appears to provide appropriate habitat for fostering the survival of rare Ozarkian species.

ACKNOWLEDGMENTS

I thank C. E. Colten, J. A. Ferguson, S. McGuire, R. B. McMillan, S. K. Santure, and K. B. Tankersley for their help with collections in the field. I am grateful to R. D. Oesch for confirming several species identifications. D. Figg, of the Missouri Department of Conservation, kindly provided information on rare and endangered species in Missouri. I thank S. Starnes of the U. S. Geological Survey in Rolla, Missouri, for supplying discharge and gage-height data. I would also like to thank A. E. Bogan, J. A. Ferguson, R. B. McMillan, J. R. Purdue, R. D. Oesch, B. W. Styles, M. D. Wiant, and two anonymous reviewers for comments on the manuscript.

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Date of manuscript acceptance: 21 January 1991

REINTRODUCTION OF THE SPINY RIVERSNAIL *IO FLUVIALIS* (SAY, 1825) (GASTROPODA: PLEUROCERIDAE) INTO THE NORTH FORK HOLSTON RIVER, SOUTHWEST VIRGINIA AND NORTHEAST TENNESSEE

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ABSTRACT

Io fluvialis was reintroduced in 1978 to two sites in the lower North Fork Holston River, along the Virginia and Tennessee border, and subsequently at one upstream site above Saltville, Virginia, in 1979. Reproduction by transplanted *I. fluvialis* was first observed in 1986 at Cloud Ford, 2.4 km downstream from the two lower transplant sites. Quantitative sampling in 1987 and 1988 resulted in population estimates of 1.75/m², which are comparable to healthy self-sustaining populations in the Clinch and Powell rivers. Qualitative sampling in 1990, at the upper transplant site also confirmed reproduction. This successful re-introduction demonstrates that transplants can be considered a viable, long-term technique for re-establishing aquatic snails in river systems that were previously polluted but are now in recovery.

The spiny riversnail, *Io fluvialis* (Say, 1825), is a large (50 mm length) freshwater prosobranch snail occurring in the Clinch, Powell, and Nolichucky rivers. Shells are generally thick, tapered at both ends, high-spired (Fig. 1), and vary in color from tan to dark brown or olive green. Shells have six or seven whorls (coils) and there is lateral wrinkling of the epidermal surface. Two phenotypes (smooth and spiny forms) are found in the tributaries of the upper Tennessee River system (Adams, 1915). Shell sculpture on the smooth form consists of folds or parallel ridges that are slightly nodulous, without pronounced spines, and the spiny form has from one to 12 (generally seven) well-developed spines.

Historically, *Io fluvialis* is endemic to the Tennessee River and was reported as far downstream as Muscle Shoals, Alabama (Lewis, 1876; Hinkley, 1906), but it was considered more common in the upper Tennessee River and larger tributaries upstream from Chattanooga (Adams, 1915; Clench, 1928; Parmalee and Bogan, 1987). With impoundment of the Tennessee River system in the 1930s and 1940s, recent mollusk studies and environmental assessments in the Tennessee River and its tributaries indicate that *I. fluvialis* was extirpated from these streams. Presently, only the headwaters of the upper Clinch and Powell rivers, and the lower Nolichucky River support native populations of *I. fluvialis*.

Industrial pollution has been a major factor in the extirpation of *Io fluvialis* from the North Fork Holston River. From

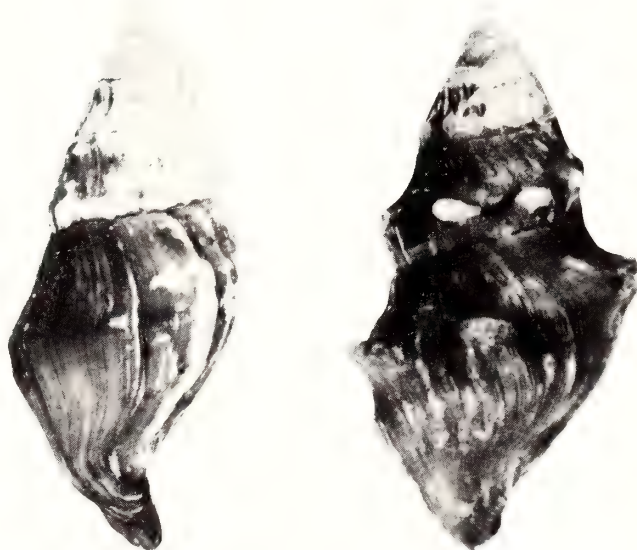


Fig. 1. Phenotypes of the spiny riversnail *Io fluvialis* (smooth-left 48.3 mm, spiny-right 47.1 mm).

1894 to 1972, the Olin Chemical Corporation maintained a soda ash plant on the banks of the North Fork Holston near Saltville, Virginia. Chloride wastes and other chemicals from this facility were discharged into the river and later into settl-

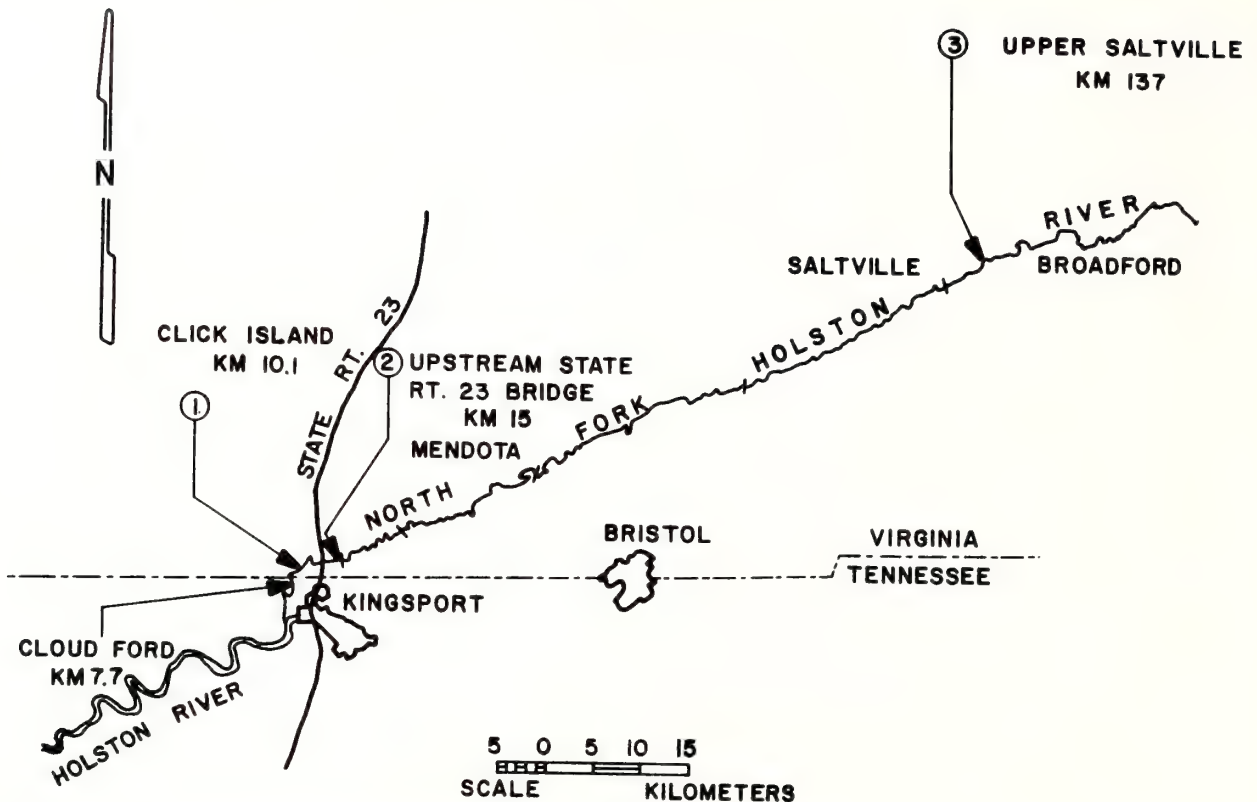


Fig. 2. North Fork Holston River Transplant Sites, 1978 and 1979.

ing lagoons (Ahlistedt, 1979). As a result of chemical discharge, aquatic organisms in the lower 128 km of the North Fork were effectively eliminated, especially freshwater mollusks (Adams, 1915; Ortmann, 1918). Adams (1915) reported collecting *I. fluvialis* in 1900 above the alkali plant upstream from Saltville, the type locality. This was the last reported sighting of *I. fluvialis* in the North Fork. The chemical plant was closed in 1972 because it could not afford to comply with water quality standards. Freshwater mussels have yet to recolonize this reach of the river (Stansbery, 1972; Stansbery and Clench, 1974; Stansbery and Stein, 1976; Ahlistedt, 1979).

From 1971 until 1976, the Tennessee Valley Authority (TVA) conducted a biological sampling program to monitor the anticipated recovery of both fish and macroinvertebrate populations in the North Fork Holston River. Routine monitoring indicated that fish and benthic macroinvertebrates were recovering rapidly. The objective of this subsequent investigation was to reintroduce and re-establish *I. fluvialis* into the North Fork.

MATERIALS AND METHODS

I. fluvialis was first reintroduced initially in July and August, 1978, at two sites in the lower North Fork Holston, and at one additional site in the upper North Fork, in October 1979. Collections of *I. fluvialis* were obtained from the upper Clinch River in July and August 1978, at the following sites

and kilometer (km) locations: Kyles Ford (km 305) and Wallens Bend (km 309), Hancock County, Tennessee. A total of 716 live specimens were collected by snorkeling and handpicking individual snails from bedrock ledges and boulder substrata. In October 1979, an additional 557 specimens were collected from the same two sites. Both the smooth and spiny forms were collected and included various size classes (13-53 mm). Specimens were transported in insulated coolers containing river water to the following locations in the North Fork Holston River: 270 specimens to Click Island (km 10.1), and 446 specimens to upstream of the State Route 23 bridge (km 15), Scott County, Virginia; and 557 specimens upstream from Saltville (km 137), Smyth County, Virginia (Fig. 2). Transport time was approximately 1 hr to the lower two transplant sites on the North Fork, and 2 hr to the upper site upstream of Saltville, Virginia. At each site, specimens were removed from the coolers and placed in nylon mesh bags anchored in riffle areas to reduce stress. Shell measurements (mm) were made with vernier calipers and included: length or height, measured from the tip of the apex to the tip of the anterior canal; and width, measured from the periphery of the last whorl behind the outer lip of the aperture to the widest spine on the opposite side of the shell. Specimens were numbered with white water-proof paint. Numbers were placed on the widest part of the shell behind the outer lip of the aperture. Small individuals were marked with an 'X'. Snails were placed by hand in creases along bedrock ledges and among boulders to

reduce passive transport in current, or to keep them from tumbling into silted areas. Because of insufficient funding and the long-term nature of monitoring the three transplant sites, every effort was made to qualitatively sample at least one site yearly, after the final transplant in 1979. Samples were collected by snorkeling, wading, and using a water scope during low-flow condition to determine whether the species was surviving and possibly reproducing.

Io fluvialis densities were determined from 1987 data using a stratified random sampling design. A total of 43/m² samples were taken with a 1/m² quadrat sampler along a transect in five areas. In each stratum, 4% of the area was sampled. Substratum within each of the 43 quadrats was visually searched and sampled by snorkeling. *I. fluvialis* collected were measured, counted, and returned to the substrata. Population estimates were calculated by the area-density method (Everhart *et al.*, 1975).

RESULTS AND DISCUSSION

During October 1986, while monitoring a transplant of the endangered birdwing pearly mussel, *Lemiox rimosus* Rafinesque, 1831 [= *Conradilla caelata* (Conrad, 1834)], two live specimens (one spiny and one smooth) of *Io fluvialis* were found in the lower North Fork Holston at Cloud Ford (km 7.7), Sullivan County, Tennessee. Neither specimen had any numerical markings or was measured (estimated to be 20-25 mm in length), and both were returned to the river. Both specimens were assumed to be the result of reproduction and downstream colonization from the original transplant of *I. fluvialis* at Click Island (km 10.1), since Cloud Ford (km 7.7) is located 2.4 km downstream of the snail transplant site. Time constraints and high water conditions prevented additional sampling for *I. fluvialis* in the North Fork in 1986. In October 1987, during quantitative sampling for *L. rimosus* at Cloud Ford, 43 quadrat samples yielded 68 specimens of *I. fluvialis* for a mean density of 1.60/m². Positive evidence of reproduction was documented since the smallest specimen recovered in October 1987 measured 12.5 mm, and the smallest specimen transplanted into the North Fork measured 13.7 mm. Resampling at Cloud Ford in October 1988, resulted in 34 *I. fluvialis* collected from 31 quadrat samples for a mean density of 1.00/m². Sampling conditions in 1988 were poor at Cloud Ford because of abundant aquatic vegetation, and extremely low water levels from a prolonged drought. Annual transplant evaluations for *L. rimosus* were not conducted by TVA at Cloud Ford during fall 1989, but large numbers of *I. fluvialis* were observed visually by the author during October 1989, while wading at Cloud Ford. Currently, populations are now estimated at 1,665 individuals or 1.75/m² at Cloud Ford. These population estimates are comparable to spiny riversnail populations sampled by the author from a number of collecting sites on the Clinch and Powell rivers.

Visits to *Io fluvialis* transplant sites at Click Island (km 10.1) and State Highway 23 bridge (km 15) in June 1989 provide only qualitative evidence of reproduction and downstream colonization in the river, based on the extent of downstream

colonization and the different sizes and numbers of individuals observed. The transplant site in the upper North Fork Holston (km 137) upstream from Saltville, Virginia had not been sampled since September 1985, when four live snails were found. All four were large adults, of which two retained partial markings of white epoxy paint. No evidence of reproduction was observed at this site. In February 1990, reproduction of *I. fluvialis* was documented at the upper transplant site on the North Fork. A total of 26 live specimens were collected qualitatively during 2.5 man-hours of sampling time. Specimens ranged in length from 10.3 - 50.7 mm. The smallest specimen, transplanted to this site in 1979, measured 18.4 mm. As judged by the large size of some of the specimens found, they are likely to be survivors from the original transplant. The white epoxy numbers marked on the shells at all three transplant sites have since worn away; however, lateral growth increments (growth rests) on some of the larger snails, especially at the upper transplant site are light-green (indication of new growth) and more pronounced than the dark green color of the shell when it was first transplanted from the Clinch River. Based on these findings, *I. fluvialis* could be relatively long-lived.

It is unknown whether *Io fluvialis* at the upper site in the North Fork is colonizing the river downstream. The first shoal, located approximately 275 m downstream, was sampled for 1 man-hour, but no *I. fluvialis* were found. Sampling was inadequate, because of high-flow conditions and deeper water habitat present at this shoal. At present, it is reasonable to assume that *I. fluvialis* will continue to reproduce and eventually colonize the upper North Fork.

CONCLUSIONS

Transplantation or reintroduction of species is one technique used successfully for a number of aquatic and terrestrial species throughout the world. Biological data indicated that the North Fork Holston River was in recovery following pollution abatement. The success of the *Io fluvialis* transplants into the North Fork confirmed the suitability of this technique for re-establishing aquatic snails in previously impacted river systems. However, establishment of a viable reproducing population through transplants is a long-term process. In the North Fork, documented evidence of successful reproduction (juveniles) did not appear until 9 years post-introduction. Population estimates in the lower North Fork Holston (Cloud Ford) are now comparable to remnant populations in the Clinch and Powell rivers. Since the two reproducing populations of *I. fluvialis* are separated by nearly 128 km of river, suitable sites between these two are recommended for additional transplants, as well as other sites in recovering streams within its historic range.

ACKNOWLEDGMENTS

I wish to thank K. Henn, R. Smith, and D. Hill for assisting with the collection and transplant of *Io fluvialis*; and C. Saylor, G. Hickman, and R. Biggins for monitoring the transplant sites.

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Date of manuscript acceptance: 17 December 1990

RESEARCH NOTE

FIRST RECORD OF NET COLLECTED *OCYTHÖE TUBERCULATA* (CEPHALOPODA: OCTOPODA) FROM PERUVIAN WATERS

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ABSTRACT

Ocythöe tuberculata Rafinesque, 1814, is recorded from net captured animals for the first time from Peruvian waters, thus confirming its presence in the southeastern Pacific Ocean. Three specimens were captured in oceanic waters at depths from 10 to 180 m. These records are consistent with the known epipelagic distribution of *O. tuberculata*.

The literature on cephalopods contains numerous records of the pelagic octopod *Ocythöe tuberculata* Rafinesque, 1814 (see review in Roper and Sweeney, 1976). Collectively, these reports suggest that *O. tuberculata* is distributed in warm waters, however, it has not been recorded from the tropics or from the southern hemisphere except for captures in the eastern South Atlantic off South Africa (Voss, 1967a) and in the far eastern Indian Ocean off Australia (Roper and Sweeney, 1976; Lu and Phillips, 1985).

As part of my current studies on the pelagic cephalopods from Peruvian waters, three net collected *Ocythöe tuberculata* were located in the collections of the Instituto del Mar del Perú (IMARPE) and the Museo de Historia Natural, Universidad Nacional Mayor de San Marcos (MUSM). These specimens represent the first net captures of *O. tuberculata* from Peruvian waters. The measurements and indices used herein are defined by Pickford and McConnaughey (1949) and Roper and Voss (1983).

Ocythöe tuberculata Rafinesque, 1814

Material examined. - 1 female, ML = 66.8 mm, R/V HUMBOLDT, Cruise 8202, 17° 02'S, 77° 42.5'W, 180 m, 988/400 mesh pelagic trawl, 24 Feb 1982, T. Dioses leg., IMARPE M46.011. - 1 female, ML = 105.0 mm, EUREKA XLVII, 14° 24'S, 77° 13'W, 30-40 m, purse seine, 27 Feb 1982, W. Elliott and M. Ñiquen leg., IMARPE M46.012. - 1 male, ML = 15.6 mm, R/V SNP-1, Cruise 8805-06, 16° 49.6'S, 72° 49.4'W, 10-40 m, 434/400 mesh pelagic trawl, 22 May 1988, A. Chipollini leg., MUSM 460101.

The specimens, typical of the species, have arm pairs

II and III much shorter than arm pairs I and IV, ventral water pores only, web greatly reduced, and a complicated mantle-funnel locking apparatus that is unique among the octopods. The ventral surface of the mantle of the females is covered by tubercles in a reticulate pattern. The much smaller male lacks the dermal tubercles and has the right arm III hectocotylized and enrolled in a membranous sac. All three specimens are sexually immature (Table 1).

Distribution. - The three specimens were taken singly in Peruvian waters, between latitudes 14° and 18°S (Fig. 1). The two females were collected in association with subtropical surface-water masses. The male was collected in association with coastal cold-water masses (21.3°C, S = 34.987 ‰). All specimens were captured with non-closing nets in oceanic waters.

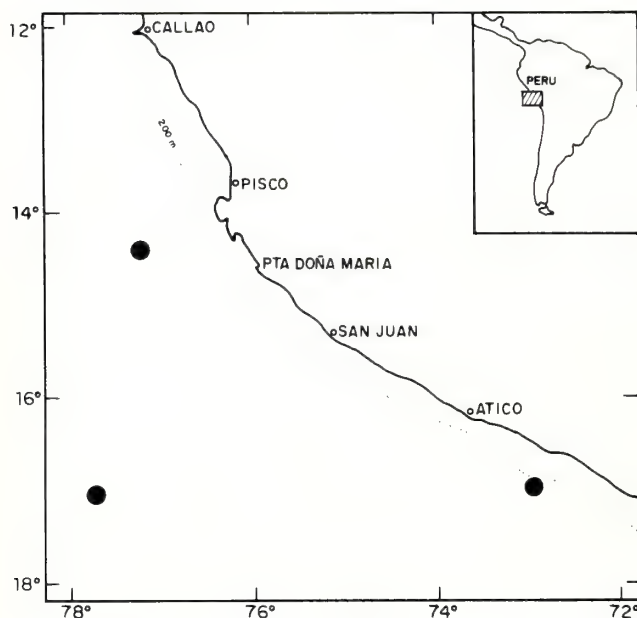
DISCUSSION

Roper and Sweeney (1976) summarized the records of *Ocythöe tuberculata* from the Mediterranean Sea, the North and eastern South Atlantic Oceans, and the North Pacific Ocean and described the first occurrence of the species from the Indian Ocean off Australia. Lu and Phillips (1985) gave the known range of *O. tuberculata* in Australian waters as from New South Wales to the Great Australian Bight. Based on analyses of stomach contents, Shchetinnikov (1986) listed *O. tuberculata* as a food item of the giant ommastrephid squid *Dosidicus gigas* from off Peru. The Peruvian specimens described here represent the first net captures of *O. tuberculata* from the eastern South Pacific Ocean.

The known depth distribution of *Ocythöe tuberculata*

Table 1. Measurements (in mm) and indices of *Ocythoe tuberculata* (a = approximate value; b = arm enrolled in sac).

Specimen	IMARPE M46.011 Female		IMARPE M46.012 Female		MUSM 460101 Male	
Mantle length	66.8		105.0		15.6	
Mantle width	34.1 ^a		63.8		11.5	
Head width	19.6		41.4		9.8	
Arm lengths	L	R	L	R	L	R
I	118.0	113.7	231.4	230.0	40.6	42.7
II	71.4	71.0	171.4	181.6	21.0	21.5
III	69.3	61.9	150.8	150.0	17.2	9.3 ^b
IV	108.7	98.4	199.2	215.0	43.4	43.8
Hectocotylized arm length	—		—		9.3 ^b	
Total length	182.2		330.0		60.6	
Mantle width Index	51.0 ^a		60.8		73.7	
Head width Index	29.3		39.4		62.8	
Arm length Index	64.8		70.1		72.3	
Mantle arm Index	56.6		45.4		35.6	
Hectocotylized Index	—		—		21.2 ^b	
No. gill lamellae	17		18		10	

Fig. 1. Localities of specimens of *Ocythoe tuberculata* from Peruvian waters.

indicates that it is limited to near-surface waters (Voss, 1967b; Roper and Young, 1975). Capture records by Roper and Sweeney (1976) from the Sargasso and the Mediterranean seas (0 to 200 m) and my data (10 to 180 m) indicate that *O. tuberculata* inhabits the epipelagic zone.

Ocythoe tuberculata is known from six areas: Mediterranean Sea; North and eastern South Atlantic Oceans; North and eastern South Pacific Oceans; southeastern Indian Ocean. It is an epipelagic and possibly cosmopolitan species in warm oceanic waters. Its occurrence in tropical waters remains unconfirmed.

ACKNOWLEDGMENTS

The author wishes to thank Dr. C. F. E. Roper, Smithsonian Institution, Dr. G. Lamas, Museo de Historia Natural, Lima, and two anonymous reviewers for offering valuable suggestions on an earlier version of the manuscript. I am also grateful to A. Chipollini, M. Niquen and T. Dioses for providing information about capture data.

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Date of manuscript acceptance: 9 May 1990

**SYSTEMATICS, ANATOMY AND EVOLUTION OF
WESTERN NORTH AMERICAN LAND MOLLUSCS**

A SYMPOSIUM IN HONOR OF WALTER B. MILLER

ORGANIZED BY

F. G. HOCHBERG AND BARRY ROTH
SANTA BARBARA MUSEUM OF NATURAL HISTORY

AMERICAN MALACOLOGICAL UNION
LOS ANGELES, CALIFORNIA
28 - 29 JUNE 1989

FAMILIAL RELATIONSHIPS AND BIOGEOGRAPHY OF THE WESTERN AMERICAN AND CARIBBEAN HELICOIDEA (MOLLUSCA: GASTROPODA: PULMONATA)

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ABSTRACT

Nordsieck's (1987) *Revision des Systems der Helicoidea*, the latest published classification of the superfamily, is further revised. By showing that anatomical characters of certain xanthonychid subfamilies are as distinctive as the ones now used to designate familial rank for other helicoid families, we reaffirm Schileyko's (1978) elevating the Humboldtianinae to familial rank and we propose raising the Helminthoglyptinae, *sensu* Nordsieck, to familial rank. We support the placing of *Monadenia* by Nordsieck into a separate subfamily and we suggest that it could belong in the Bradybaenidae.

The discrete anatomical characters of the American helicoid families and their discontinuous geographical distribution bring into question the theory of a putative continuous radiation and evolution from an Asian origin. Recent geophysical data have shown that large parts of eastern Asia and western America were formed from Gondwanian terranes that migrated tectonically from the south Pacific Ocean. We suggest that most helicoid families, Asian and American, arrived at their separate destinations, passively, via these Pacifican terranes.

The classification of the land snail superfamily Helicoidea, formerly called Helicacea, has been the subject of many studies and revision within the past 50 years. Pilsbry (1939) provided the first concise key which designated the distinguishing familial characters of the Helicidae, Helicellidae, Bradybaenidae, Helminthoglyptidae, Polygyridae, Camaenidae, and Sagdidae, all of which he included in the Helicacea. Zilch (1959-1960) removed the Polygyridae and the Sagdidae from the Helicoidea and placed them in the superfamily Polygyracea (now Polygyroidea, in accordance with Article 29a of the *International Code of Zoological Nomenclature*). Solem (1978) further revised the Helicoidea by placing the Camaenidae in its own superfamily. The most recent revision of the Helicoidea was made by Nordsieck (1987) in which he recognizes the following families: Sphincterochilidae Zilch, 1960; Xanthonychidae Strebel and Pfeffer, 1880; Bradybaenidae Pilsbry, 1934; Hygromiidae Tryon, 1866; Helicidae Rafinesque, 1815.

In considering the familial relationships within a superfamily, one is naturally led to speculate on the evolution of these families from a single ancestral group and their

dispersal from some point of origin to their present distribution. The most generally accepted theory on the biogeography of the Helicoidea has been that the ancestral helicoid probably arose in the Palearctic during the Mesozoic era (Pilsbry, 1894, 1939) from whence the ancestors of the European helicoids could easily spread throughout Europe while the ancestral xanthonychids invaded the Americas via the Bering land bridge no later than early Eocene and probably earlier (Pilsbry, 1894).

We have been studying the biogeography of the western North American helicoids for many years, especially in northwest Mexico, and we have been puzzled by their total absence from a large part of Sonora where a continuous radiation would have been expected from a trans-Beringean southward radiation. We have also been puzzled by the total absence of helicoids from a large part of northwestern South America which would have had to be traversed in order to reach Peru and Argentina.

Recent determinations in geophysics that most of eastern Asia and a large part of Northwest America were not part of the Asian continent or the American continent,

respectively, during the Mesozoic, but rather were part of Gondwanian land masses located in early Mesozoic in the south-central Pacific Ocean led us to investigate the possibility that Asian and western American and Caribbean helicoids could have arrived at their present destinations via the Gondwanian terranes from a Pacifican origin. Our concurrent studies on the familial characteristics of the American helicoids have also shown that several of Nordsieck's xanthonychid subfamilies are sufficiently different, anatomically, to warrant familial status. Together, the pronounced anatomical differences and the discontinuous geographical distributions of these subfamilies have provided a compelling stimulus to investigate available evidence which could support a theory of a Pacifican origin for the superfamily. Detailed analyses and resulting determinations follow.

FAMILIAL RELATIONSHIPS

Pilsbry (1939) placed all Western American and Caribbean Helicoidea into the single family Helminthoglyptidae. He recognized no less than eight strongly differentiated groups which he ranked as subfamilies. Only seven, however, were named: Helminthoglyptinae Pilsbry, 1939; Sonorellinae Pilsbry, 1939; Humboldtianinae Pilsbry, 1939; Cepoliinae Pilsbry, 1939 (emendation for Cepolinae Hoffman, 1928, a homonym); Xanthonychinae Strebel and Pfeffer, 1879 (as Xanthonychidae); Lysinoinae Hoffman, 1928; Epiphragmophorinae Hoffman, 1928. Of these seven subfamilies, Zilch (1959-1960) lumped Lysinoinae into Humboldtianinae and Epiphragmophorinae into Helminthoglyptinae. Subsequently, Schileyko (1978) raised Humboldtianinae to familial rank.

The use of the name Helminthoglyptidae was brought into question by Baker (1959) when he showed that the name Xanthonychidae Strebel and Pfeffer, 1879 had considerable priority over Helminthoglyptidae Pilsbry, 1939. Furthermore, Baker showed in a precise chronological review of family names from 1867 to 1958 that the Old World family Bradybaenidae had been considered repeatedly to include many New World subfamilies of the Xanthonychidae. Indeed there were no precise, consistent characters that could be used to separate Bradybaenidae from Xanthonychidae. Nevertheless, Baker condescended to allow the use of the name Bradybaenidae with this statement (1959): "Since the sizes of families are matters of convenience and/or custom, we Americans, North and South, can leave to the wisdom of our Old World colleagues the advisability of a separate family for the genera of their home lands". To date, European malacologists have continued to use the name Bradybaenidae for what Baker considered to be "Old World Xanthonychidae", while many American malacologists have continued to use the name Helminthoglyptidae for New World Xanthonychidae.

All helicoid families, Old World as well as New World, are characterized by having a reproductive system equipped with a dart and mucus gland apparatus associated with or in close proximity to the vagina; certain genera in this superfamily without a dart apparatus are believed to have become secondarily simplified in their evolution from dart-bearing ancestors. The principal characters used in separating families

within the Helicoidea are the type and shape of the mucus glands and the position of their insertion into the vagina or the dart sac. Pilsbry (1939) published a simple key of distinguishing characters to differentiate each family which can be summarized as follows:

I. HELICIDAE: medium or large snails, usually with banded shells, having one dart sac with two tubular, simple or branching mucus glands inserted close to its base, the spermatheca on a long duct which usually bears a branch.

II. HELICELLIDAE (now in Hygromiidae): of medium or small size, with the dart sac often twinned, sometimes wanting, the tubular mucus glands when present inserted well above it on the vagina; spermathecal duct medium or short, never branching.

III. HELMINTHOGLYPTIDAE (now Xanthonychidae): dart sac or sacs and mucus glands present, the latter club-shaped, globular or irregular (not tubular or finger-shaped), inserted close to the base of the dart sac.

Pilsbry then briefly referred to the Bradybaenidae "of Eastern Asia" as having irregular type mucus glands opening through an accessory sac on the dart sac or sometimes directly at the base of the latter.

It was clear from the above definitions that Pilsbry's Helminthoglyptidae and Bradybaenidae were a catch-all group for those species whose mucus glands were not tubular or finger-shaped. Schileyko (1978) reviewed the superfamily Helicoidea, with emphasis on anatomical characters. He recognized that the Humboldtianinae formed a consistently distinct group characterized by four compact dart sacs arranged circumferentially high on the vagina, and four globular, compact mucus glands also arranged circumferentially above the dart sacs and inserting directly into the vagina at the level of the dart sacs. He therefore raised this subfamily to familial rank as a distinct and separable taxon whose character differences were of equal magnitude as the character differences used in separating the Helicidae and Hygromiidae. Miller (1987) agreed with Schileyko and showed that the genus *Bunnya* Baker, 1942, was characterized by a similar set of dart sacs and mucus glands, except that their number was three instead of four, and thus this genus should be included in the Humboldtianidae.

Most recently, Nordsieck (1987) published a revision of the taxonomy of the Helicoidea based on a detailed study of the dart apparatus and mucus glands of the various groups. He illustrated diagrammatically the various configurations of the system and defined each of the numerous families, subfamilies, and tribes, many of them newly erected. He then prepared an elaborate cladogram based not only on the dart apparatus and mucus glands but also on the presence or absence of accessory seminal vesicles, chromosome number, presence or absence of accessory dart sacs, presence or absence of spermathecal diverticulum, and the position of the spermatheca (either along the spermoviduct or bent away from it.).

The main thrust of Nordsieck's cladogram, however, was to concentrate on the evolution of the Helicidae, Hygromiidae, and Bradybaenidae. A similar detailed analysis would have been highly desirable for the Xanthonychidae,

although it is recognized that there are large gaps in our knowledge of anatomical characters and chromosome numbers of the numerous xanthonychid subfamilies. Nordsieck affirmed that the correct familial name for the Western American and Caribbean Helicoidea should be Xanthonychidae as Baker (1959) had shown, and he recognized the following nine subfamilies in the Americas: Monadeniinae Nordsieck, 1987; Helminthoglyptinae Pilsbry, 1939; Cepoliinae Pilsbry, 1939; Epiphragmophorinae Hoffman, 1928; Trichodiscininae Nordsieck, 1987; Lysinoiinae Hoffman, 1928; Xanthonychinae Strebel and Pfeffer, 1880; Metostracinae Nordsieck, 1987; Humboldtianinae Pilsbry, 1939. By returning the Humboldtianinae to the Xanthonychidae as a subfamily, he disagreed with Schileyko's familial ranking for this group. He concurred that *Bunnya* belonged with the Humboldtianinae and erected the new tribe Bunnyiini for this genus.

Nordsieck's revision of the Helicoidea provides the most authoritative classification of the superfamily at this time. Disagreements with his findings must be substantiated with evidence. In addition, the dictates of the International Code of Zoological Nomenclature must be followed if we are to avoid chaos in nomenclature.

During the past many years, we have been examining the reproductive anatomies of most of the numerous genera of the western North American helicoids and, in many cases, most or all of their individual species. The genera most thoroughly examined are the following: *Helminthoglypta* Ancey, 1887; *Micrarionta* Ancey, 1880; *Xerarionta* Pilsbry, 1913; *Eremarionta* Pilsbry, 1913; *Plesarionta* Pilsbry, 1939; *Monadenia* Pilsbry, 1895; *Sonorella* Pilsbry, 1900; *Sonorelix* Berry, 1943; *Mohavelix* Berry, 1943; *Tryonigens* Pilsbry, 1927; *Greggelix* Miller, 1972; and *Eremariontoides* Miller, 1981. Additional genera also critically examined but from only a few representative species are the following: *Epiphragmophora* Doring, 1875; *Averellia* Ancey, 1887; *Humboldtiana* Ihering, 1892; *Lysinoe* H. and A. Adams, 1855; *Cepolis* Montfort, 1810; *Bunnya* Baker, 1942.

In all of our examinations, we were impressed repeatedly by certain distinguishing characters that stood out markedly from all others and were consistently occurring in the species of what are now classified by Nordsieck as three subfamilies, namely the Humboldtianinae, the Helminthoglyptinae (*sensu* Nordsieck, i.e. minus *Monadenia* which had been included by Pilsbry), and the Monadeniinae. In the genus, *Humboldtiana*, all of our species had four compact, vesicular mucus glands, circumferentially arranged around the vagina and inserting directly into it; additionally, they had four dart sacs also circumferentially arranged around the vagina and located immediately below the mucus glands. This arrangement was strikingly unlike any other system found in any other helicoid except *Bunnya*. In the Helminthoglyptinae, all dart bearing species had one or both mucus glands consisting of wide membranes that wrapped around various parts of the anterior end of the reproductive tract such as the penis, and/or the vagina, and/or the atrial sac. Again, this arrangement was strikingly different from any other helicoid system. Finally, in the Monadeniinae, all species were equipped with only a single, tubular mucus gland which inserted on a large swelling

of the anterior end of the reproductive tract, a structure somewhat resembling the accessory sac (Nordsieck's Nebensack) found in some of the Bradybaenidae. Although Pilsbry (1939) considered the anterior muscular swelling to be simply an atrium, the similarity with the bradybaenid accessory sac could not be ignored; furthermore, the tubular mucus gland would, by Pilsbry's own definition, exclude this subfamily from the Xanthonychidae.

We came to the conclusion, therefore, that the anatomical differences found in the Humboldtianinae and in the Helminthoglyptinae were as distinctive and of equal or greater magnitude as those now used to designate familial rank for other helicoid families, namely Helicidae, Hygromiidae, Bradybaenidae, and Sphincterochilidae. Accordingly, we concur with Schileyko that the Humboldtianinae should be raised to familial rank. We further propose that the Helminthoglyptinae, *sensu* Nordsieck, also be raised to familial rank. Because Miller (1970, 1973, 1981) already provided evidence that certain genera in Pilsbry's Sonorellinae (Nordsieck's Sonorellini) probably arose from different helminthoglyptine ancestors, namely *Mohavelix* Berry from *Helminthoglypta micrometalleoides* Miller, 1970, and *Eremariontoides* Miller from *Eremarionta greggi* Miller, 1981, the Sonorellini must be considered polyphyletic and unacceptable as a taxon. To date, there are no convincing data available to indicate the immediate dart-bearing ancestors of *Sonorella* Pilsbry, *Sonorelix* Berry, *Greggelix* Miller, and *Tryonigens* Pilsbry. Classification of the helminthoglyptid genera at the subfamilial level, therefore, will have to await more sophisticated methods of analysis probably involving chromosome banding and DNA hybridization. Finally, we also concluded that the Monadeniinae were more closely similar, anatomically, to the Bradybaenidae than to the Xanthonychidae, Humboldtianidae, or Helminthoglyptidae so that they should be classified either as a bradybaenid subfamily or raised to familial rank. At this time, we recommend the conservative approach of leaving them in subfamilial status as a fourth bradybaenid subfamily along with Bradybaeninae Pilsbry, 1924, Aegistinae Kuroda and Habe, 1955, and Helicostylinae Ihering, 1909.

A simple key can be erected as follows to separate the Humboldtianidae and the Helminthoglyptidae from the Xanthonychidae:

1. One or more mucus glands membranous Helminthoglyptidae
Mucus glands vesicular 2
2. Mucus glands and dart sacs compact, multiple, seated high on the vagina Humboldtianidae
Mucus glands and dart sacs not thus Xanthonychidae

Additionally, figure 1 shows the most probable phylogeny of the families, based on the concept that membranous mucus glands are probably ancestral to vesicular glands.

In summary, the classification of the Western American and Caribbean Helicoidea, lumped by Nordsieck (1987) into the single family Xanthonychidae, is now proposed as follows:

Helicoidea Rafinesque
 Xanthonychidae Strebel and Pfeffer
 Cepoliinae Pilsbry
 Epiphragmophorinae Hoffman
 Trichodiscininae Nordsieck
 Lysinoinae Hoffman
 Xanthonychinae Strebel and Pfeffer
 Metostracininae Nordsieck
 Humboldtianidae Pilsbry
 Humboldtianinae Pilsbry
 Bunnyinae Nordsieck
 Helminthoglyptidae Pilsbry
 Bradybaenidae Pilsbry
 Monadeniinae Nordsieck

BIOGEOGRAPHY

After determining that the anatomical characteristics of the Western American and Caribbean Helicoidea were sufficiently distinct to separate at least four different families, namely Xanthonychidae, Bradybaenidae, Humboldtianidae, and Helminthoglyptidae, our attention turned to their possible evolution and ultimate dispersal from a common origin. Figure 2 shows the general distribution of the Western American and Caribbean Helicoidea and their East Asian relatives. To account for this nearly circum-Pacific distribution, many terrestrial malacologists theorized that these helicoids had a Eurasian origin and migrated into the Americas via a Bering land bridge during the Tertiary period (Pilsbry, 1894; Pilsbry, 1948; Gregg, 1959). This theory seemed to us to leave too many important questions unanswered. For example, in order to attain the current distribution in the Americas (Fig. 2), the theory presumed a long and narrow dispersal along the west coast of North America into Mexico and Central America followed by a radiation eastward to the main Caribbean islands as well as many of the lesser Antilles, ultimately as far as the Bahamas and southern Florida. In the meantime, however, these helicoids failed to reach the central and eastern United States although there is ample evidence that during that same time period the Polygyridae were able to populate extensive areas from southern Florida to New England to Washington, Oregon, and northern California and nearly all of central and western Mexico. The Helicoidea also would have crossed the isthmus of Panama to reach vast areas of Peru and northwestern Argentina but they failed to leave any trace in Panama, Colombia, and Ecuador; they also failed to disperse into Venezuela and Brazil. More recently, as a result of a five year study of helminthoglyptid distribution in Sonora, Mexico, by one of us (ENG), we found a complete absence of helminthoglyptids from the latitude of Hermosillo to the Sinaloa border (Naranjo-García, 1988); yet we found that region to be well populated by other families of large snails such as bulimulids and polygyrids. Such gaps in distribution, unexplainable by geological or ecological events due to the presence of other families of large snails in these gaps, presented a serious flaw in the theory of a Bering land bridge migration.

During the past four decades, several biogeographers

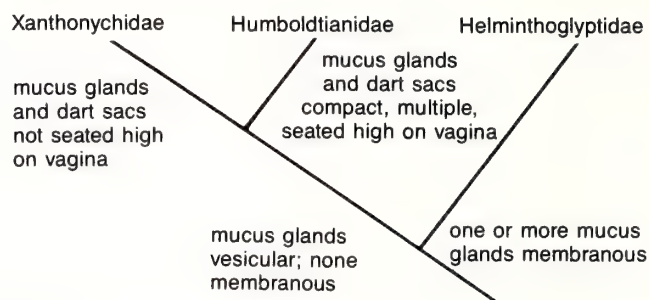


Fig. 1. Probably phylogeny.

began to question the widely invoked theories of paleartic origins for much of the New World biota. Croizat (1952) and Melville (1966, 1981) suggested that the exchange of biota through a Bering land bridge was relatively insignificant. Melville (1966), in order to explain the distribution of angiosperms along the Pacific Rim, proposed the existence of a mesozoic land mass in the south-central Pacific, which he named Pacifica, that broke up and migrated tectonically to accrete to the continental margins of Asia and America. Nur and Ben-Avraham (1977) suggested that the circum-Pacific mountain belts could have been the result of past continental collisions similar to those associated with the Alpine belt. They proposed that these collisions were made by parts of a continental mass situated in the South Pacific Ocean, Pacifica (referring to Melville's 1966 article), which disaggregated during the Mesozoic and spread out on the Kula, Farallon, Phoenix, and Pacific plates eventually to reach continental margins. Subsequently, additional geophysical and geological evidence appeared in the literature in support of the former existence of Pacifica (Kamp, 1980; Davis *et al.*, 1978; Coney *et al.*, 1980; McGeary and Ben-Avraham, 1981; Nur and Ben-Avraham, 1982). Then Jones *et al.* (1982) showed convincing evidence, supported by the work of Tarduno *et al.* (1986), that western North America consists of accreted terranes which originated thousands of kilometers to the south and west of their present position. Jones *et al.* also provided evidence to show that Permian terranes, originally formed in the Tethys Sea, had also accreted to form a large part of eastern Asia. Kulm *et al.* (1986), while studying the subduction zone of Oregon and Washington which possesses accreted terranes, found communities of clams and tube worms (*Calypptogena* sp.) similar to those found in the accretionary complexes of Japan, the Philippine Plate, and other locations around the Pacific Ocean. Figure 3 is a schematic composite model of Pacifica land mass migrations during the Mesozoic era, according to these cited authors.

In the light of this mounting volume of evidence, we studied the possibility that the circum-Pacific Ocean distribution of the Helicoidea could be much better explained by theorizing a center of origin on a Mesozoic Pacifican land mass, Pacifica, which broke up into several parts that were ultimately carried, as Nur and Ben-Avraham suggested, to form large parts of western North and South America and east Asia. These terranes provided a passive means of dispersal for ancestral populations of helicoids to dock at various

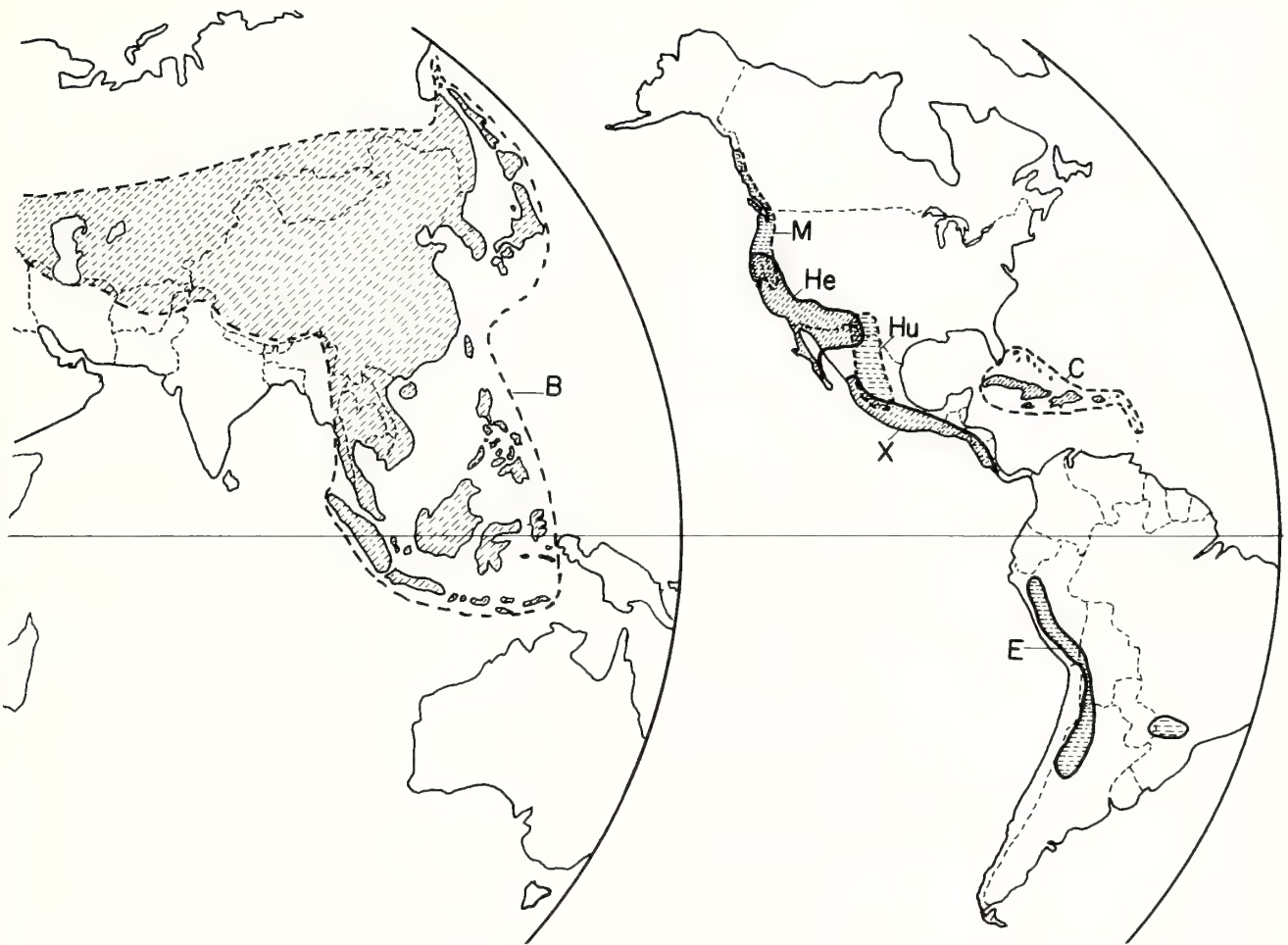


Figure 2. Approximate areas of distribution of Western American and Caribbean Helicoidea and Asian Bradybaenidae: B, Bradybaenidae (other than Monadeniinae); C, Cepoliinae (Xanthonychidae); E, Epiphragmophorinae (Xanthonychidae); He, Helminthoglyptidae; Hu, Humboldtianidae; M, Monadeniinae (Bradybaenidae); X, Xanthonychidae (other than Cepoliinae and Epiphragmophorinae).

discrete parts of the Americas and Asia. With this theory, the absence of any Helicoidea from Panama, Colombia, Ecuador, and a large area of Sonora did not need to be attributed to mass extinctions. Moreover, the time period involved in the break-up and migration of the Pacifican terranes during the Mesozoic would have permitted ample isolation for the evolution of separate families and subfamilies on each different terrane.

We came to the conclusion that the Pacifican theory did indeed better explain the circum-Pacific distribution of the helicoids than the trans-Beringean theory. To explain the current distribution of the families and subfamilies of these helicoids, we hypothesize the following vicariance patterns: 1) the arrival of the ancestral Bradybaenidae in eastern Asia, with subsequent dispersal along the shore of the Tethys Sea to south-central Asia and into southern Europe; 2) the arrival of the ancestral Helminthoglyptidae in western North America, docking along what is now California and Baja California, with eventual dispersal eastward as far as west Texas and Chihuahua; 3) the arrival of the ancestral Xanthonychidae, other than Epiphragmophorinae and Cepoliinae, along the

shores of southwestern Mexico and Central America; 4) the arrival of the ancestral Epiphragmophorinae in the vicinity of Peru, with eventual dispersal southeasterly as far as northwest Argentina and southern Brazil; 5) the arrival of the ancestral Cepoliinae into the Caribbean region on the Greater Antilles terranes which Burke *et al.* (1984) stated had origins in the Pacific Ocean during the Mesozoic and migrated to collide with the Bahamas long before the formation of the Isthmus of Panama.

In the case of the Humboldtianidae, conchological and anatomical characters indicate a closer evolutionary relationship to the European helicoids than to the other American helicoids (Schileyko, 1978). Additionally, their widespread distribution not only over the entire Mexican Plateau but also, as fossils, as far north as east-central Wyoming led us to hypothesize that they must have been indigenous to the North American craton after it separated from Laurasia. They are, therefore, the one American helicoid family that apparently did not evolve from Pacifican ancestors.

Although the theory of a Pacifican origin for the East Asian, Caribbean, and Western American helicoids (except-

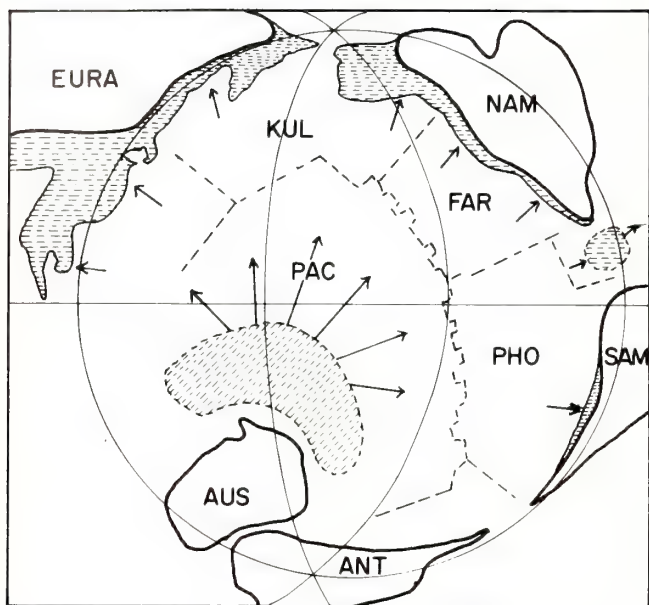


Figure 3. Schematic composite model of estimated Pacifica land mass migrations during Mesozoic era. Heavy lines mark estimated positions of continental areas at end of Mesozoic, according to Dietz and Holden (1975). Horizontal dashed lines mark presumed Pacifican terranes that accreted to the continents according to Jones *et al.* (1982) and Burke *et al.* (1984). Diagonal dashed lines mark estimated position of Pacifica at beginning of Mesozoic according to Nur and Ben-Avraham (1977). Major Pacific plates during Mesozoic according to Zonenshain *et al.* (1984): ANT, Antarctica; AUS, Australia; EURA, Eurasia; FAR, Farallon plate; KUL, Kula plate; NAM, North America; PAC, Pacific plate; PHO, Phoenix plate; SAM, South America.

ing the Humboldtianidae) satisfactorily explains the distribution of the current populations, it does not rule out the possibility of some limited Tertiary trans-Beringean migration. As stated earlier, we consider the Monadeniinae to be more closely related to the Bradybaenidae than to any other American helicoid group. The possibility exists, therefore, that this bradybaenid subfamily could have migrated across the Bering land bridge from northeast Asia. Conchological characters would support a very close relationship between Japanese *Euhadra* species and *Monadenia* species. Anatomically, however, the Monadeniinae are very different from Japanese *Euhadra* or from northeast Siberia *Bradybaena*. Accordingly, it appears to us that the ancestral Monadeniinae evolved on a separate Pacifican terrane that ultimately docked on the North American continent somewhere on the Canadian or southern Alaskan coast.

ACKNOWLEDGMENTS

We are especially indebted to Barry Roth for making us aware of the Pacifica theory and suggesting that our helicoids could have originated there. Moreover, he assisted greatly in bringing to our attention the fact that *Monadenia* appeared to have greater affinity with the Bradybaenidae as well as in providing the key and the cladogram used in this article to differentiate between the Xanthonychidae,

Helminthoglyptidae, and Humboldtianidae. We also wish to thank both Eric Hochberg and Barry Roth for their encouragement and their helpful suggestions in the preliminary review of this manuscript. The Consejo Nacional de Ciencia y Tecnología provided grant support for the studies in Sonora by one of us (ENG).

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Date of manuscript acceptance: 26 July 1990

A PHYLOGENETIC ANALYSIS AND REVISED CLASSIFICATION OF THE NORTH AMERICAN HAPLOTREMATIDAE (GASTROPODA: PULMONATA)

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ABSTRACT

A revised classification of the North American Haplotrematidae (=Haplotrematinae) is presented, based on character compatibility analysis of 25 characters of the musculature, reproductive system, radula, kidney, pericardium, and shell. Two genera are recognized -- *Ancotrema* and *Haplotrema*, the latter with three subgenera, *Ancomena*, *Geomene*, and *Haplotrema*, *sensu stricto*. The hypothesized age of the Haplotrematinae is 42.5 million yr (late middle Eocene), when the temperate Haplotrematinae and its tropical out-group, the Austroselenitinae, are assumed to have diverged. The phylogenetic hypothesis indicates an early dichotomy between *Ancotrema* and *Haplotrema*, with the species of *Ancotrema* retaining more characteristics of the hypothetical ancestor. Other evolutionary trends include: (a) a tendency toward reduction of vaginal musculature; (b) a parallel tendency toward greater penial complexity; (c) migration of the origin of the penial retractor muscles from the columellar muscle bundle to the floor of the lung (presumably increasing mechanical efficiency); (d) parallel instances of gigantism in the Pacific Northwest; (e) size reduction southward along the Pacific Coast, correlating with the presence/severity of a summer dry season; (f) a homoplastic continuation of the loss or reduction of cusps on the teeth of the radula. A lectotype is designated for *Selenites vancouverensis* forma *hybrida* Ancy, 1888.

The Haplotrematidae are a specialized family of rhytidoidean land snails restricted to the Western Hemisphere. Baker (1941) divided the family into the subfamilies Haplotrematinae (North American) and Austroselenitinae (Antillean and South American). The Haplotrematinae constitute a holophyletic group (*sensu* Ashlock, 1971), based on characters of the kidney, ureter, sigmoid loops of the hindgut, salivary gland conformation, jaw, and radula. In the current classification (Pilsbry, 1946), which draws heavily on the detailed anatomical studies of Baker (1931, 1941), all species of Haplotrematinae are assigned to the genus *Haplotrema* Ancy, 1881. [The classification of Zilch (1960) merely strews all previously proposed genera and subgenera of Haplotrematidae as subgenera under the genus *Haplotrema*.] Table 1 compares the classifications of Baker (1931) and Pilsbry (1946), both of which used the non-standard category "section," with the classification proposed in this paper.

Since the time of those authors' writings, methods have been developed that enable systematists to analyze patterns of character variation in accordance with stated criteria in order to develop hypotheses about the evolutionary history of groups of organisms. The purpose of this study is to apply

some of these methods to derive a classification of the species of Haplotrematinae that is supported by as many independent characters as possible and therefore represents the best available estimate of the phylogenetic relationships of these taxa. The method used is character compatibility analysis (Estabrook, 1972; Le Quesne, 1982), which has its roots in the observations of Wilson (1965) and Le Quesne (1969, 1972). Character compatibility analysis is the method of choice where, as here, one seeks the tree exhibiting the most congruence in the synapomorphy pattern of the taxa under study (Strauch, 1984). The analysis incidentally provides a test of how well the characters studied and utilized by Baker (1931, 1941) support his own classification.

This inquiry grew out of my preparation of: (a) a treatment of the Haplotrematidae for the Council of Systematic Malacologists "Checklist of the Non-marine Mollusca of the United States and Canada" (Pratt, in prep.) and (b) an identification manual of the land snails and slugs of California for the California Departments of Food and Agriculture and Fish and Game. This analysis and discussion are beyond the scope of either of those works, and so are presented in this paper.

Table 1. Comparison of classifications of the North American Haplotrematidae (=Haplotrematinae).

Baker (1931)	Pilsbry (1946)	This Paper
Genus <i>Haplotrema</i> Ancey, 1881	Genus <i>Haplotrema</i>	Genus <i>Haplotrema</i>
subg. (<i>Haplotrema</i> , s.s.)	subg. (<i>Haplotrema</i> , s.s.)	subg. (<i>Haplotrema</i> , s.s.)
sect. <i>Haplotrema</i> , s.s.	sect. <i>Haplotrema</i> , s.s.	subg. (<i>Geomene</i>)
sect. <i>Geomene</i> Pilsbry, 1927	sect. <i>Geomene</i>	subg. (<i>Ancomena</i>)
subg. (<i>Ancotrema</i>) Baker, 1931	subg. (<i>Ancomena</i>)	Genus <i>Ancotrema</i>
sect. <i>Ancotrema</i> , s.s.	sect. <i>Ancomena</i> , s.s.	
sect. <i>Ancomena</i> Baker, 1931	sect. <i>Greggiella</i> Baker, 1941	
	sect. <i>Ancotrema</i>	

MATERIALS AND METHODS

Character compatibility analysis (Estabrook, 1972, 1978) was used to identify patterns of agreement and disagreement among characters in the data set and to identify which characters were most useful in constructing an estimate of the phylogenetic relationships among the 16 valid specific and infraspecific taxa assigned to the Haplotrematinae. This analysis was performed manually according to the method described by Meacham (1981), and subsequently using the Fortran program CLINCH by Kent L. Fiala, with identical results. Examples of the use of character compatibility analysis include Duncan (1980) and Meacham (1980), where further references to its theoretical and mathematical basis can be found.

The basic structure of the phylogenetic hypothesis was established from the primary clique(s) of characters derived through compatibility analysis. Then rejected characters and character state trees were re-evaluated for the additional information they could provide.

Anatomical data are lacking for some of the taxa of the Haplotrematinae. Four taxa regarded by previous authors as species either have not been dissected or the available anatomical information is incomplete. They are therefore excluded from the analysis: tentative suggestions for their placement are given in the discussion. The subspecific relationship between *Haplotrema durantei durantei* (Newcomb, 1864) and *H. d. continentis* (Baker, 1931) is maintained, although only shell and radular data are available from *H. d. durantei*. One other taxon is regarded as a *species inquirenda*.

Sources of data are listed below. The abbreviations BR and TP designate locality numbers from the collections of the author (San Francisco, California) and of Timothy A. Pearce (Ann Arbor, Michigan), respectively. In addition to the anatomical material cited, approximately 900 lots of shells were examined, mainly in the Santa Barbara Museum of Natural History, California Academy of Sciences, and the author's collection.

TAXA INCLUDED AND DATA SOURCES

alameda Pilsbry, 1930. Baker (1931); author's dissection of nominal subsp. *fieldi* Pilsbry, 1930, from Kern River canyon, 1.6 km southwest of Democrat Hot Springs, Kern County, California (BR 957).

caelatum (Mazzyck, 1886). Baker (1931, 1941).

concaum (Say, 1821). Baker (1931), Pilsbry (1946);

additional information on genitalia from Webb (1943). Subsp. *minus* (Ancey, 1882) is a synonym (Pilsbry, 1946).

duranti (Newcomb, 1864). Radula from topotypic subsp. *duranti* (Baker, 1931); other anatomy based on *H. d. continentis* Baker, 1931 (Baker, 1931).

hybridum (Ancey, 1888). Baker (1931); author's dissection from Cape Blanco, Curry County, Oregon (BR 1173). Figure of genitalia by Webb (1961) shows some characters as in *Ancotrema sportella*, others equivocal [e.g. bulge on left side of vagina labeled "pilaster" resembles muscular collar found in this position in *A. sportella* (Gould, 1846) and other species]. Although Baker (1931) reported intergradation between "the *semidecussatum* form of *hybridum*" and *A. sportella* in western Washington, in northwest California and southwest Oregon *hybridum* occurs at the same localities as typical *A. sportella* and *Haplotrema vancouverense* (Lea, 1839) without intergradation. Lectotype designation in a following section fixes type locality as Astoria, Oregon.

"Form" *semidecussatum* Gratacap is not an available name. The original publication (as *Macrocyclis vancouverensis* [Lea] var. *semi-decussata*) was not intended as the proposal of a new taxon but merely the reference to a museum label (apparently from Thomas Bland) accompanying "a very interesting specimen...from Astoria, Oregon" (Gratacap, 1901:340). The text goes on to quote the opinion of H. A. Pilsbry that "it should not be accorded position and is probably var. *hybrida* Ancey" (Gratacap, loc. cit.). Baker (1931) provided diagnostic information but treated it as infrasubspecific "form" of "*Haplotrema sportella hybridum*." The anatomy is reported to be the same as in *A. hybridum* (Baker, 1931).

"Var." *depressa* Ancey, 1888, is an unavailable infrasubspecific name.

keepi (Hemphill, 1890). Baker (1931); Webb's (1961) figure of genitalia of specimen from Dog Creek, Shasta County, California, and author's dissection from Signal Butte, Shasta County (TP 861026-1215), differ in characters of the penis and spermathecal duct and could represent a different, undescribed species.

minimum (Ancey, 1888). Baker (1931); author's dissections from Duncan Point, Sonoma County (BR 1466); Point Reyes Peninsula, Marin County (BR 1490, BR 1640, BR 1642); San Pablo Dam Road above San Pablo Reservoir, Contra Costa County (BR 316); Sharps Park, San Mateo County (BR 104); Malpaso Creek, Monterey County (BR 1503), California. "Forms" *occidentale* (Hemphill In: W. G. Binney, 1892),

tenuis (Hemphill In: W. G. Binney, 1892), and *kelseyi* (Hemphill, 1911) are synonyms (Pilsbry, 1946).

sportella (Gould, 1846). Baker (1931); author's dissections from along Woods Creek, west of Philomath, Benton County, Oregon (BR 1681); Moonstone Beach, Humboldt County, California (TP 831228-1). Figures of genitalia by Webb (1961) corroborate some characters. Porter's (1965, 1968) figures of the genitalia of a specimen from near Corvallis, Oregon, show an extremely short, saccular spermathecal duct with a pointed end, not otherwise reported in the species. A specimen that I dissected from Woods Creek, about 15 km west of Corvallis (BR 1681), has the elongate spermathecal duct and globose spermatheca normal in the species. Perhaps Porter's (1965, 1968) material was abnormal, or the structures were misinterpreted.

transfuga (Hemphill In: W. G. Binney, 1892). Baker (1941).

vancouverense (Lea, 1839). Baker (1931); author's dissections from Brookings, Curry County, Oregon (BR 357); Luffenholtz Beach, south of Trinidad, Humboldt County, California (BR 354). Figures of genitalia by Webb (1961) and Porter (1965, 1968) add no new information. "Forms" *vellicatum* (Forbes, 1850) and *chocolatum* (Dall, 1905) are synonyms (Pilsbry, 1946), although a lectotype designation for *chocolatum* may be needed to confirm this (cf. remarks by Henderson 1936:257).

voyanum (Newcomb, 1865). Baker (1931, 1941); author's dissection from Bidden Creek, Trinity County, California (BR 1229).

TAXA NOT INCLUDED IN THE ANALYSIS

catalinense (Hemphill In: W. G. Binney, 1890). Not dissected.

costatum Smith, 1957. Not dissected.

guadalupense Pilsbry, 1927. Not dissected.

humboldtense Pilsbry, 1946. Based on material that Baker (1931) included in *H. voyanum*, *Haplotrema voyanum humboldtense* Pilsbry, 1946, was described in such vague terms and with such a generalized type locality ("Humboldt and 'Klamath' counties," Calif.) that even a meaningful search for the type population would be difficult. Whether Klamath County, Oregon, was meant (Baker, 1931:421) or the former California county of the same name, as in Pilsbry's interpretation, is not known. Until such time as the type material can be matched up with a population somewhere, *H. v. humboldtense* must remain a *species inquirenda*.

kendeighi Webb, 1951. Originally described as a subspecies of *Haplotrema concavum*; regarded as a species by Hubricht (1956, 1985). Dissection by Hubricht (1956) incompletely described.

CHARACTERS

Appendix A lists the characters, character states, and hypothesized character state trees used in the analysis. Characters of the musculature, reproductive system, radula, kidney, pericardium, and shell are included. No weighting was assigned *a priori* to the characters of any one system.

The polarity of character state transformations was inferred mainly on the basis of out-group comparison at various levels of generality, and in some cases by correlation of equivocal characters with the direction of other, better-grounded transformations. For example, the reduction of radular tooth secondary cusps (apomorphic states of characters 3-5) parallels a well-established, general trend in carnivorous land snails and slugs toward a "slicing" or "stabbing" tooth morphology (Watson, 1915; Solem, 1974). Some use was made of the assumption that the most common state of a character is primitive. The Haplotrematidae have no significant fossil record, so no inferences could be based on stratigraphic precedence.

Direction of "chorological progression" — suggested by Hennig (1966) as one method of inferring polarity — could be based, for example, on the observation that the Klamath Mountains are a refugium preserving vegetation (Whittaker, 1961; Axelrod, 1976) and perhaps a land snail fauna (Roth, 1981) more like that of the later Cenozoic than any other region in the west. This would lead to the hypothesis that character states found in *Haplotrema voyanum* and *H. keepi*, residents of the Klamath region, are primitive within their clades. However, one of the uses of a phylogenetic hypothesis is to illuminate the biogeographic history of a group, and excessive reliance on this form of reasoning would introduce circularity into a biogeographic analysis. Chorological progression therefore was considered only in the spirit of "reciprocal illumination" (Hennig, 1966; Page, 1987).

Table 2 shows the distribution of character states among the 12 taxa included in the analysis. A hypothetical ancestor, with all characters in the inferred primitive condition, is added. Its character states are not necessarily those of the Austroselenitinae, because the species of that tropical group have specializations of their own [for example, the slender, elongate atrium of *Austroselenites (Zophos) concolor* (Férussac, 1820) (Baker, 1941:pl. 9, fig. 6)]. Comments on specific characters follow.

The right ocular retractor is free from the genitalia (character 1, state B) in all Austroselenitinae dissected (Baker, 1931, 1941). Posterior position of the external genital orifice (character 2, state B) is an apomorphy of the Haplotrematinae (and perhaps Haplotrematidae) in general, but plesiomorphous within the group. *Haplotrema* generally enter the shell of a prey snail through the aperture or through a hole broken just behind the peristome (pers. observ.); relocation of the genital apparatus backward probably allows the head and foreparts to reach farther inside the shell. A genital orifice close to the base of the right ommatophore (characters 2, state A) in *Haplotrema durante* and *H. caelatum* probably represents a space-related reversal in these small-sized species. On Santa Barbara Island, California, *H. durante* preys on the pupillid snail *Nearctula rowelli* (Newcomb, 1860), entering the shell through the side of the whorls of the spire (F. G. Hochberg, pers. comm., 1979); it probably does not require the extreme elongation of the foreparts needed by species that go up the body whorl of their prey.

Character 4 (multiple/reduced number of cusps on central tooth of radula) is kept separate from character 5

Table 2. Distribution of character states among species of North American Haplotrematidae and hypothetical ancestor.

Taxon	Symbol	Character No.				
		1-5	6-10	11-15	16-20	21-25
<i>caelatum</i>	CAE	AABBB	CAABA	AABAA	ACBAB	BAABB
<i>concamum</i>	CON	ABABA	CAABA	BABBB	ACBAB	BBDBA
<i>duranti</i>	DUR	AAABA	CAABB	BABBB	ACBAA	ABCBB
<i>hybridum</i>	HYB	BBAAA	AAABB	AAABB	BAABC	ABAAA
<i>keepi</i>	KEE	BBABA	CAAB	CABBB	BCBCA	ABDBA
<i>minimum</i>	MIN	BBABA	CABBB	AABBB	BABAA	ABDBA
<i>alameda</i>	ALA	BBABA	CAABB	BABAB	ABBAA	ABBBB
<i>sportella</i>	SPO	BBAAA	AAABB	AAABB	BAABC	ABAAB
<i>transfuga</i>	TRA	BBABB	CAABB	BABAB	ABBAA	ABBBB
<i>vancouverense</i>	VAN	BBABA	CBBBB	ABBBB	BACAA	ABDBA
<i>voyanum</i>	VOY	BBAAA	BAABB	AAABB	BABAC	ABAAB
ancestor	ANC	BBAAA	AAABB	AAABB	BAAAC	ABAAA

(presence/absence of cusps on central tooth) because multiple cusps could be lost without passing through a stage of reduction in number. Migration of the origin of the penial retractor muscle from the columellar bundle to the floor of the lung (character 6) probably represents a transition toward greater mechanical efficiency. A tendency for the main penial chamber to be differentiated into apical and basal parts (character 13) is also seen in the out-group Austroselenitinae.

RESULTS

PRIMARY CLIQUES

Two primary cliques of 17 characters each were derived. The cladograms based on these cliques are shown in figure 1. Symbolic abbreviations of species names used in the figures and in the following discussion are given in Table 2. Characters 3, 7, 8, 9, 12, 15, and 22 are compatible with all others in the analysis (all but character 8 are characters unique to one analyzed species) and are therefore common

to both primary cliques. In addition to these "consensus" characters, both cliques include characters 1, 4, 6, 10, 13, 18, 19, 21, and 24. The two cliques differ only in the presence of character 16 (clique A) or character 17 (clique B). The components of cladograms A and B grouping (ANC, ((SPO, HYB), VOY)) and (DUR, (CON, CAE)) are isomorphous in both cladograms. The cladogram based on clique A, with character 16 (atrium with/without stimulator), contains an unresolved trichotomy. The cladogram based on clique B, with the multi-state character 17 (vagina with shining muscular collar/with sphincteric thickening/without collar or thickening), is more fully resolved and is accepted as the best estimate of actual patterns of ancestry and descent in the Haplotrematinae.

OTHER CHARACTERS

2. An external genital orifice close to the right omatophore in DUR and CAE is a probable homoplasy associated with small size.

5. Absence of cusps on the central tooth of the radula

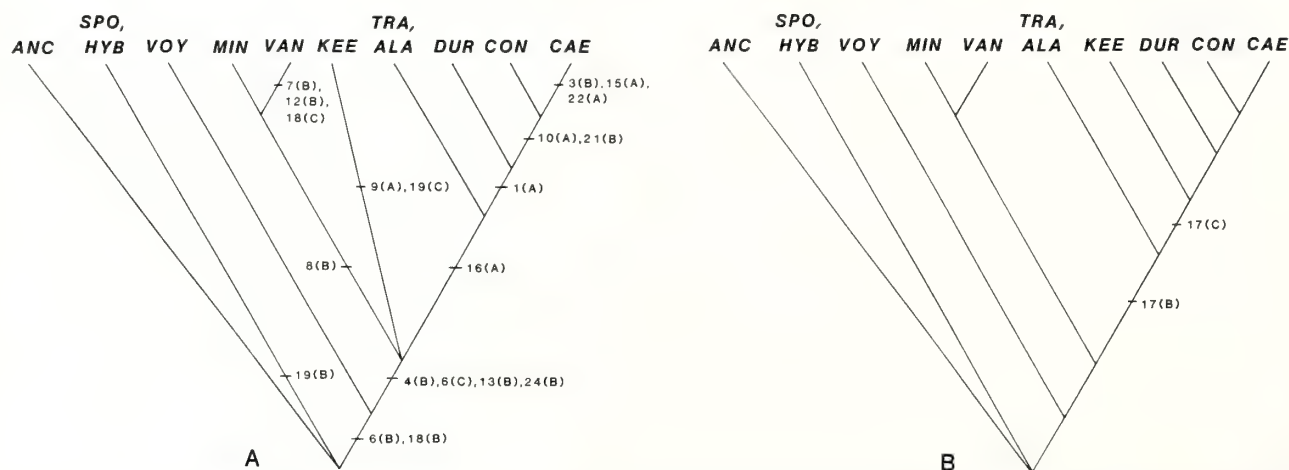


Fig. 1. Cladograms based on the two primary cliques: (A) based on clique with character 16; (B) based on clique with character 17. Abbreviations of taxa as in Table 2. Numbers on branch segments designate apomorphic states of characters as defined in Appendix (shown in B only where different than in A).

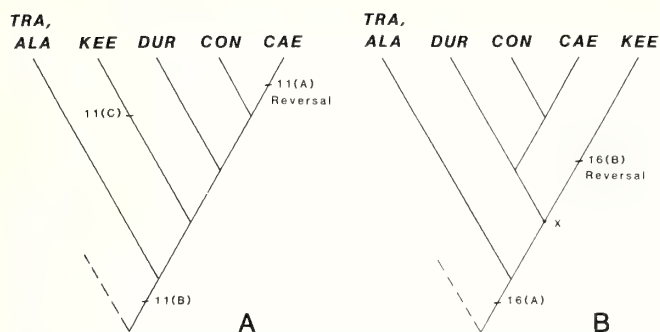


Fig. 2. The right-hand branch of cladogram B (from Fig. 1) with inferred apomorphies and reversals of character 11 (A) and character 16 (B). Component (KEE,(DUR,(CON,CAE))) is rotated around node x in right diagram for clarity; the order of branching remains unchanged.

is a homoplasy, occurring independently in TRA and CAE.

11. Sharp papillae are present on the penial wall of (TRA,ALA), DUR, and CON; blunt papillae are present in KEE. As shown in figure 2, derivation of blunt papillae from sharp, and one reversal (to papillae absent in CAE), are sufficient to incorporate this character into the basic cladogram.

14. A penial sheath is present in CAE and (TRA,ALA); homoplastic origins are indicated.

16. A stimulator is present in the atrium in (TRA,ALA) and (DUR,(CON,CAE)) but absent in KEE. As shown in figure 2, a single reversal is sufficient to incorporate this character into the basic cladogram.

20. The relative length of the kidney is greatest in ((MIN,VAN),(TRA,ALA),KEE,DUR) and least in ((SPO,HYB),VOY); a partial reversal involving (CON,CAE) is indicated. When recoded C → A → B, this character becomes compatible with the primary clique.

23. A basic trend from broad to narrow radial ribs is established but the absence of ribs is homoplastic. Unribbed shells apparently have been derived from shells with broad, narrow, or threadlike ribs. The presence of broad ribs in CAE represents at least a two-step reversal.

25. Minute, wavy spiral striation may be generally distributed through the Haplotrematinae but its expression masked on ribbed shells. Its distribution in the group studied approximately parallels that of smooth shells, which is homoplastic.

DISCUSSION

Figure 3 presents the hypothesis of the phylogeny of the Haplotrematinae produced by this study. Five characters from five systems (radula, musculature, kidney, reproductive system, and shell) support the dichotomy between the group (ANC,((SPO,HYB),VOY)) and the rest of the subfamily. The former group consists of the species that Baker (1931) referred to the nominate "section" of *Ancotrema*. Neither primary clique contains an apomorphy defining *Ancotrema* or distinguishing it from the hypothetical ancestor; the group is paraphyletic. A plausible alternative coding of character 24,

with state A (coarse spiral striae cutting tops of radial ribbing) as apomorphic, would distinguish *Ancotrema* but is not compatible with characters 6 and 18. Similarly, round-topped radial ribs wider than their interspaces could be apomorphic in *Ancotrema*.

An alternative, less parsimonious interpretation, which would allow VOY to be grouped with (SPO,HYB) on the basis of these shell characters, is that migration of the penial retractor muscle to the floor of the lung (character 6, states B, C) and elongation of the vagina (character 18, states B, C) are homoplastic. Obsolescence of radial ribs on the penult and body whorls distinguishes HYB from SPO. In conjunction with increased size, it is a noteworthy convergence upon VAN and occurs in the same region — the moist forests of the Pacific Northwest — where other cases of gigantism in land mollusks are known.

Chromosome number, although known for only two species of the Haplotrematinae and, therefore, not entered in the compatibility analysis, supports the distinction between *Ancotrema* and *Haplotrema*. The haploid chromosome number of VAN is 30, that of SPO, 29 (Burch, 1965). The higher number is considered to be apomorphic.

Four characters, all having to do with the reproductive system, distinguish (MIN,VAN) from ((TRA,ALA),(KEE,(DUR,(CON,CAE)))). (MIN,VAN) is defined by the autapomorphy of a swollen vas deferens (character 8, state B). This group contains the type species of *Ancomena* (VAN, *Haplotrema vancouverense*) and is here recognized as a restricted subgenus *Ancomena* of *Haplotrema*. The other species included in *Ancomena* by Baker (1931), TRA, ALA, and KEE, are removed from that subgenus. TRA is distinguished from ALA by the absence of a cusp on the central tooth and by minor quantitative differences in shell sculpture and genitalia.

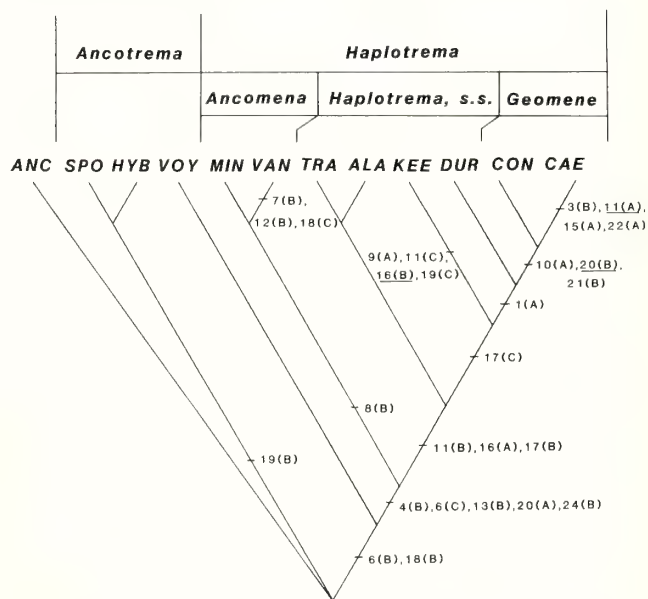


Fig. 3. Phylogenetic hypothesis of the Haplotrematinae, and genus/subgenus boundaries. Underlining denotes reversal; other conventions as in Fig. 1.

The remaining species are a rather heterogeneous lot, although with a clearly hierarchic set of relations. It is easy to see why Baker (1931) and Pilsbry (1946) resorted to three-layered classifications using the infrasubgeneric category "section," but not so easy to decide on a single set of rules for dividing this array into subgenera. (CAE, CON) is holophyletic, with three defining apomorphies. The subgenus name *Geomene*, based on CON, has priority over *Greggiella*, based on CAE. However, four characters distinguish CAE from CON. KEE is holophyletic and distinguished by five characters, including four autapomorphies. My decision has been to recognize no monotypic subgenera and to accept two heterogeneous subgenera (in addition to *Ancomena*) in *Haplotrema* — *Haplotrema*, *sensu stricto*, and *Geomene*. Table 3 presents a revised classification of the Haplotrematinae based on this analysis.

PLACEMENT OF TAXA NOT ANALYZED

catalinense. Differs from *Haplotrema durante* principally in larger size (diameter 5.7-6.3 mm versus 5.2 mm). Fine and partly obsolete ribbing occurs also in several mainland populations of *H. d. continentis*. Dissection of both *H. catalinense* and *H. d. durante* is needed. *Haplotrema s.s.*

costatum. Character of ribbing suggests relationship to *Haplotrema caelatum*, but shell sculpture is not diagnostic in subgenus *Geomene*. Subgenus uncertain.

guadalupense. Differs from *Haplotrema durante* mainly in absence of ribbing and smooth surface. *Haplotrema s.s.*

kendeighi. Probable sibling species of *Haplotrema con-*

cavum. Hubricht (1956) distinguished it from *H. concavum* by the color of the foot and by the large atrium, "at least twice the size of that of [*H. concavum*]," even in smaller animals. None of the reported characters associates *H. kendeighi* with any West Coast species of *Haplotrema*. *Geomene*.

HISTORY OF THE HAPLOTREMATINAE

Chambers (1987) calculated the average chromosomal rate of evolution for land pulmonates at 0.021 karyotypic changes per lineage per million yr. This figure is derived from the fossil record of land snail genera (Chambers, 1987), which (a) represents *minimum* maximum ages and (b) is far more fragmentary than that of marine genera. The "short-weighting" that results from these features is somewhat counterbalanced by the tendency for paleontological systematics — based, necessarily, on shell characters alone — to construe genera broadly and by the frequency of convergence in pulmonate shell form.

After excluding *Haplotrema* (= *Ancotrema* + *Haplotrema* of this study) from Chambers's data set to avoid circularity, and recalculating the rate of karyotypic changes based on a recent Cenozoic time scale (Berggren *et al.*, 1985), using mean rather than maximum ages for the fossils of an epoch or sub-epoch, the data suggest an approximate age of 42.5 million yr (late middle Eocene) for the Haplotrematinae. It must be stressed that this estimate is based solely on an average derived from a wide variety of land pulmonates.

Fossil evidence indicates that during the middle Eocene a diverse land mollusk fauna including genera of present-day tropical distribution existed at middle latitudes in North America (Roth, 1984, 1988; Roth and Megaw, 1989). By the late middle Eocene many of these genera had begun to retreat southward and a latitudinal stratification of land mollusk faunas had become evident. The dichotomy between the temperate Haplotrematinae and its tropical out-group, the Austroselenitinae, could have arisen at this time.

The phylogenetic hypothesis indicates an early dichotomy between *Ancotrema* and *Haplotrema*, with the species of *Ancotrema* retaining more characteristics of the hypothetical ancestor. Other trends include: (a) a tendency toward reduction of vaginal musculature as one moves toward the right side of the cladogram in figure 3; (b) a parallel tendency toward greater penial complexity; (c) migration of the origin of the penial retractor muscles from the columellar muscle bundle to floor of lung (presumably increasing mechanical efficiency); (d) parallel instances of gigantism in the Pacific Northwest; (e) size reduction southward along the Pacific Coast (partly, but not wholly, on islands) correlating with the presence/severity of a summer dry season; (f) a homoplastic continuation of the loss or reduction of cusps on the teeth of the radula.

The dichotomy between the widespread eastern North American species, *Haplotrema concavum*, and a West Coast species (the highly derived *H. caelatum*) occurs higher on the tree (i.e. later in time) than the origins of all other major West Coast lineages. The vicariance was probably not related to the Laramide orogeny (which would require condensing most

Table 3. Revised classification of the North American Haplotrematidae.

Superfamily Rhytidoidea Pilsbry, 1895
Family Haplotrematidae Baker, 1925
Subfamily Haplotrematinae Baker, 1925
Genus <i>Haplotrema</i> Ancey, 1881
Subgenus <i>Haplotrema</i> , <i>sensu stricto</i>
<i>H. (H.) durante</i> (Newcomb, 1864)
<i>H. (H.) d. durante</i>
<i>H. (H.) d. continentis</i> Baker, 1931
<i>H. (H.) catalinense</i> (Hemphill <i>In</i> : W. G. Binney, 1890)
<i>H. (H.) guadalupense</i> Pilsbry, 1927
<i>H. (H.) keepi</i> (Hemphill, 1890)
<i>H. (H.) alameda</i> Pilsbry, 1930
<i>H. (H.) transfuga</i> (Hemphill <i>In</i> : W. G. Binney, 1890)
Subgenus <i>Geomene</i> Pilsbry, 1927
<i>H. (G.) concavum</i> (Say, 1821)
<i>H. (G.) kendeighi</i> Webb, 1951
<i>H. (G.) caelatum</i> (Mazýck, 1886)
Subgenus <i>Ancomena</i> Baker, 1931
<i>H. (A.) vancouverense</i> (Lea, 1839)
<i>H. (A.) minimum</i> (Ancey, 1888)
Subgenus uncertain
<i>H. costatum</i> Smith, 1957
Genus <i>Ancotrema</i> Baker, 1931
<i>A. sportella</i> (Gould, 1846)
<i>A. hybridum</i> (Ancey, 1888)
<i>A. voyanum</i> (Newcomb, 1865)

of haplotrematine evolutionary history into the later Eocene) but to a later climatic event, such as the late Tertiary emergence of an arid environment in the American Southwest (Axelrod, 1979) or Pleistocene glacio-pluvial changes, which were relatively mild in coastal southern California (Johnson, 1977) but profound in the continental interior. Several other characteristically "eastern" taxa such as the genera *Stenotrema* Rafinesque, 1819 (Polygyridae), and *Hendersonia* Wagner, 1905 (Helicinidae), were present in the North American cordillera as recently as early (and possibly middle) Miocene time (Berry, 1953; Roth and Emberton, unpub. data), but the timing of their extinction in the cordillera is uncertain.

LECTOTYPE DESIGNATION FOR *SELENITES VANCOUVERENSIS* FORMA *HYBRIDA* ANCEY, 1888

Three localities were cited in the original publication of *Selenites vancouverensis* forma *hybrida* Ancey, 1888: "Oregon, dans la region du Fleuve Columbia; Portland, Oregon (Dore); territoire de Washington." Type material was not specified. Baker (1931) designated Portland as the type locality. C. F. Ancey's collection was dispersed by the shell dealer Geret (Dance, 1966). One syntype, purchased from Geret by S. Stillman Berry in the early years of this century, is now in the Santa Barbara Museum of Natural History. On a printed label of "Geret, Conchyliologiste/Naturaliste" an unknown hand gives the locality as Astoria, Oregon. A second hand has written in pencil, "TYPE". The specimen is a mature shell, 24.2 mm in maximum diameter, 11.0 mm in height, with 6.2 whorls, agreeing with the original description but slightly lower-spired than Ancey's figure. The specimen, SBMNH 35134 (Fig. 4), is here designated lectotype of *Selenites vancouverensis* forma *hybrida*. The type locality of the taxon is therefore Astoria, Clatsop County, Oregon, rather than Portland as designated by Baker (1931).

Ancey (1888) stated that he was maintaining the name "*hybrida*" under which J. H. Thompson had designated the taxon in his private collection. The name seems to have had even wider currency among amateur malacologists of the time. Hemphill (1890a) listed "*Selenites concava* var. *hybrida*" in a sales catalog and, apparently unaware of Ancey's (1888) proposal, described the same taxon as *Selenites van-*

couverensis var. *hybrida* (Hemphill, 1890b), likewise based on specimens from Astoria (Coan and Roth, 1987).

ACKNOWLEDGMENTS

I am grateful to Paul Scott and Eric Hochberg for help with type and other specimens from the S. S. Berry collection at the Santa Barbara Museum of Natural History. Tim Pearce loaned specimens from his collection. David R. Lindberg (University of California Museum of Paleontology) provided the CLINCH analysis. Messrs. Pearce and Lindberg read drafts of the manuscript. Walt Miller discussed the results with me. Ellen and George Moore collected *Ancotrema sportella* in the Corvallis area in response to my request. I thank the anonymous reviewer who provided the HENNIG86 maximum-parsimony analysis.

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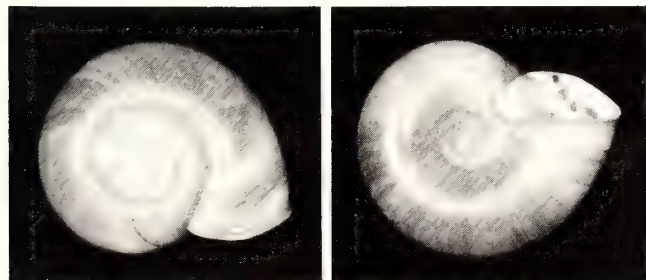


Fig. 4. Lectotype of *Selenites vancouverensis* forma *hybrida* Ancey, 1888, SBMNH 35134; top and basal views. Diameter 24.2 mm.

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Note added after revision. One anonymous reviewer of this paper performed a maximum-parsimony analysis (Farris, 1983; Kluge, 1989) of this data set using the program HENNIG86 (Farris, 1988). Four equally (and maximally) parsimonious trees were derived. One tree was isomorphous with the tree shown in figure 3; a second differed in having the branch leading to KEE and that leading to (TRA,ALA) reversed. Two other trees contained the grouping (((MIN,VAN),KEE),(TRA,ALA),(DUR,(CON,CAE)))). A classification based on this additional analysis might include KEE in the subgenus *Ancomena* and treat (TRA,ALA) as a group coordinate in rank with (DUR,(CON,CAE)).

Date of manuscript acceptance: 20 June 1990

APPENDIX A. LIST OF CHARACTERS, CHARACTER STATES, AND CHARACTER STATE TREES

Character No. 1. Right ocular retractor: in penioviducal angle (A), free from genitalia (B); B → A.

Character No. 2. External genital orifice: close to base of right ommatophore (A), distant from right ommatophore (B); B → A.

Character No. 3. Radula: with several lateral teeth bicuspid (A), with one lateral tooth bicuspid (B); A → B.

Character No. 4. Central tooth of radula: with multiple cusps (A), with reduced number of cusps (0-1) (B); A → B.

Character No. 5. Central tooth: with cusps (A), lacking cusps (B); A → B.

Character No. 6. Penial retractor muscle origin: on left side of columellar muscle (A), on floor of lung with strands from columellar bundle (B), on floor of lung (C); A → B → C.

Character No. 7. Penial retractor muscle insertion: at summit of penis with vas deferens entering laterally (A), on vas deferens, which enters penis apically (B); A → B.

Character No. 8. Vas deferens: slender throughout (A), with swollen, epiphallus-like portion (B); A → B.

Character No. 9. Penis: with flagelloid caecum (A), without (B); B → A.

Character No. 10. Penis: with diverticulum (A), without (B); B → A.

Character No. 11. Penial chamber wall: without papillae (A), with sharp, thornlike papillae (B), with blunt papillae (C); A → B → C.

Character No. 12. Apical penial chamber: well differentiated (A), not well differentiated (B); A → B.

Character No. 13. Principal penial chamber: differentiated into apical and basal parts (A), not differentiated thus (B); A → B.

Character No. 14. Penial sheath: present (A), absent (B); B → A.

Character No. 15. Penial chamber: with apical papilla (A), without (B); B → A.

Character No. 16. Atrium: with "stimulator" (A), without (B); B → A.

Character No. 17. Vagina: with shining muscular collar (A), with sphincteric thickening (although not a muscular collar) (B), without collar or thickening (C); A → B → C.

Character No. 18. Vagina: extremely short (i.e., spermathecal duct origin directly above atrium) (A), somewhat less than to approximately equal to length of penis (B), 1.5 times length of penis (C); A → B → C.

Character No. 19. Base of spermathecal duct: weakly dilated (A), strongly dilated (B), with a dual dilation (C); B → A → C.

Character No. 20. Length of kidney: 1.5 times its base (A), approximately 2 times its base (B), over 2 times its base (C); C → B → A.

Character No. 21. Length of pericardium: approximately $\frac{2}{3}$ length of kidney (A), approximately $\frac{1}{2}$ length of kidney (B); A → B.

Character No. 22. Embryonic shell: with radial riblets on last $\frac{1}{2}$ whorl (A), smooth throughout (B); B → A.

Character No. 23. Shell: with distinct, round-topped radial ribs as broad or broader than interspaces (A), with round-topped radial ribs narrower than interspaces (B); with fine, threadlike to laminar radial ribs (C), without ribs (except for low undulations of shell surface) (D); D → A → B → C.

Character No. 24. Shell: with coarse spiral striae cutting tops of radial ribbing (A), without (B); A → B.

Character No. 25. Shell: with minute, close, wavy spiral striae (A), without (B); A → B.

PRESENT STATUS OF THE MICROMOLLUSKS OF NORTHERN SONORA, MEXICO

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ABSTRACT

Information about the terrestrial micromollusk fauna of Sonora State is compiled. Fifteen genera and 22 species are reported to occur. Preliminary field work has added new locality records, as well as two species new to the state: *Euconulus fulvus* (Müller, 1774), and *Lamellaxis* sp. There are seven widely distributed species in the state. *Thysanophora hornii* (Gabb, 1866) is very well represented. The distribution of *Gastrocopta ashmuni* (Sterki, 1898) enlarges the Southwestern Molluscan Province southward to central Sonora. *Karolus consobrinus primus* De Folin, 1870, a Mid-western Mexican Province species, has affinities to the south and penetrates south and central Sonora.

RESUMEN

Se conjunta la información sobre la micro malacofauna terrestre del Estado de Sonora, se han registrado 15 géneros y 22 especies en el estado. Trabajo de campo de prospección ha permitido añadir nuevos registros de localidades, lo mismo que dos nuevas especies para el estado: *Euconulus fulvus* (Müller, 1774), y *Lamellaxis* sp. Hay siete especies ampliamente distribuidas. *Thysanophora hornii* (Gabb, 1866) es la mejor representada. *Gastrocopta ashmuni* (Sterki, 1898) extiende hacia el sur a la Provincia Malacológica del Suroeste, en el centro de Sonora, *Karolus consobrinus primus* De Folin, 1870, es un elemento de la Provincia del Oeste Medio de México que penetra el sur y centro de Sonora.

The micromollusks of Mexico have received little attention. Because of their small size they are difficult to locate and to collect. Recent explorations have increased our knowledge of the malacofauna of Sonora. Early works, which include some locality records from Sonora deal primarily with faunas of other regions. Other information is widely scattered throughout the literature. Dall (1896), in his report for the International Boundary Commission, included microgastropods from various places in Mexico except Sonora. Drake (1953, 1956), who conducted some surveys in the state, added five new records. Furthermore, he mentioned an almost completed monograph on the non-marine mollusks of the region and wrote of a collection placed in the Instituto de Biología, México, but I have not been able to locate either. Drake did not give a faunal listing for Sonora. In a paper on terrestrial snails from northern Mexico, Pilsbry (1953) cited eight records from Sonora. Branson *et al.* (1964) wrote a note on new localities of snails, which included *Vallonia perspectiva* Sterki, 1893 as a new record for the state. Bequaert and Miller (1973) discussed mainly the Arizona molluscan fauna from a biogeographical viewpoint. Their work included the Sonoran

fauna shared with Arizona. An important contribution of that work was the updating of the nomenclature of the included taxa.

Habitat information is scant and scattered, although some highlights are given in the literature, e.g. Pilsbry (1948) discussed the general features of the environment where pupillids are found. Bequaert and Miller (1973) gave additional details for several species. The other works cited above did not discuss habitat.

The purpose of this study is to present information on the distribution of microgastropods of Sonora, based on material collected from 1983 to 1988. I have tried to review most literature references, provide a point of departure for other investigations, and contribute toward the condensation of the widely dispersed information concerning the Mexican terrestrial mollusk fauna, in general.

The state of Sonora is located in the northwest of Mexico, adjacent to the Gulf of California; approximately 26° to 32°N and 108° to 115°W. It is bordered on the west by the Gulf of California, to the north by Arizona, to the east by Chihuahua state and to the south by Sinaloa state. Plains and mountains

show several biotic communities that Brown (1982) summarized for the region. The largest biome in Sonora is the Sonoran Desert which occupies most of the western half of the state. The southern and southeastern regions bear the Sinaloan Thornscrub. The Sinaloan Deciduous Forest extends from the south to the north in a narrow strip, bordered to the east by Madrean Evergreen Woodland (Gentry, 1982), and to the north by the Chihuahuan Desert and the Madrean Evergreen Woodland.

METHODOLOGY

While searching for *Sonorella* (macromollusks) (Naranjo-García, 1988) several microgastropods were found coincidentally; therefore, the majority of the samples are from central and north Sonora sites inhabited by this genus (from the Madrean Evergreen Woodland; Lower Colorado River Subdivision of the Sonoran Desert, Semidesert Grassland, to the Chihuahuan Desertscrub). In addition, an exploration to the south yielded several samples from that region. Samples were taken from the same *Sonorella* locality or a place close by. Leaf litter, humus, soil accumulations, and drift

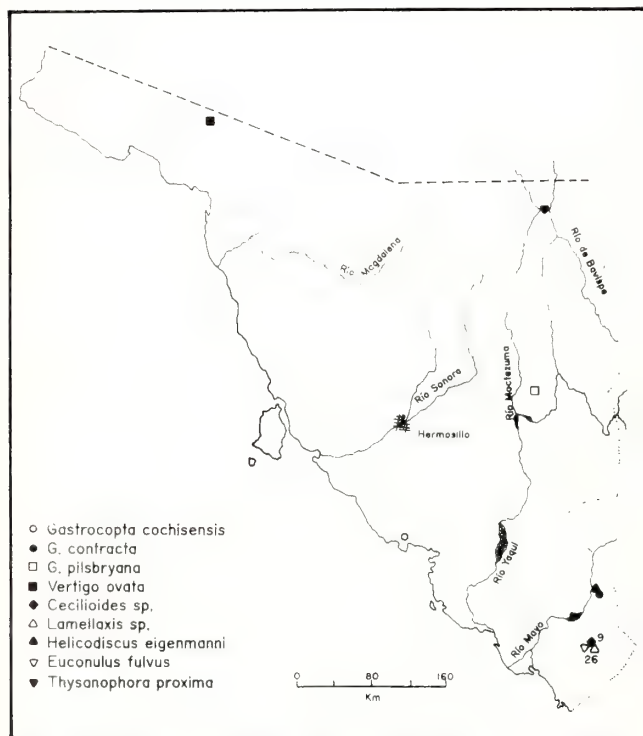


Fig. 1. Species with a single record for Sonora, numbers in the map are my localities (see Appendix). Localities of species shown in this map are from the following authors: *Gastrocopta contracta* Arroyo San Rafael, San Bernardo (Pilsbry, 1953:161); *G. cochisensis* Arroyo 8 km S from Guaymas (Pilsbry, 1953:162); *G. pilsbryana* Brushy area in mountains 29 mi. S of Cumpas Hwy 10 (Branson *et al.*, 1964:104); *Vertigo ovata* Rio Sonoyta at Sonoyta (Bequaert and Miller, 1973:183); *Helicodiscus eigenmanni* San Bernardino (Pilsbry, 1948:630); *Thysanophora proxima* Rio Bavispe 21 mi. S Agua Prieta (Branson *et al.*, 1964:103).

wash material were examined for micromollusks, then sampled. In the laboratory, soil samples were sifted by a series of sieves (3.36 mm, 1.68 mm, and 0.84 mm) to separate the different sizes of shells.

As a result of these investigations, new localities are added for the microsnail fauna of Sonora. Figures 1 to 5 present the distribution of the species known for Sonora.

RESULTS AND DISCUSSION

A total of 15 genera and 22 species have been documented for Sonora (Table 1). The primary source of data for this list of species is from the papers of Pilsbry (1948 and 1953) and Bequaert and Miller (1973); some localities shown in the maps are from those authors and from Branson *et al.* (1964). The systematics follows the classification suggested by Solem (1978), Burch and Jung (1988), and Abbott and Boss (1989). Thirty-six new localities have been added to the records (see Appendix).

The species taken in this survey have all been recorded previously from Sonora, except for two taken in my field samples that are new species records for the state: *Lamellaxis* sp. and *Euconulus fulvus* (Pilsbry and Ferriss, 1906).

FAMILY PUPILLIDAE

Gastrocopta (Immersidens) ashmuni (Sterki, 1898) (Fig. 4) is extensively distributed in Arizona, New Mexico, trans-Pecos Texas, and Chihuahua (Bequaert and Miller, 1973). It was considered by those authors as representative

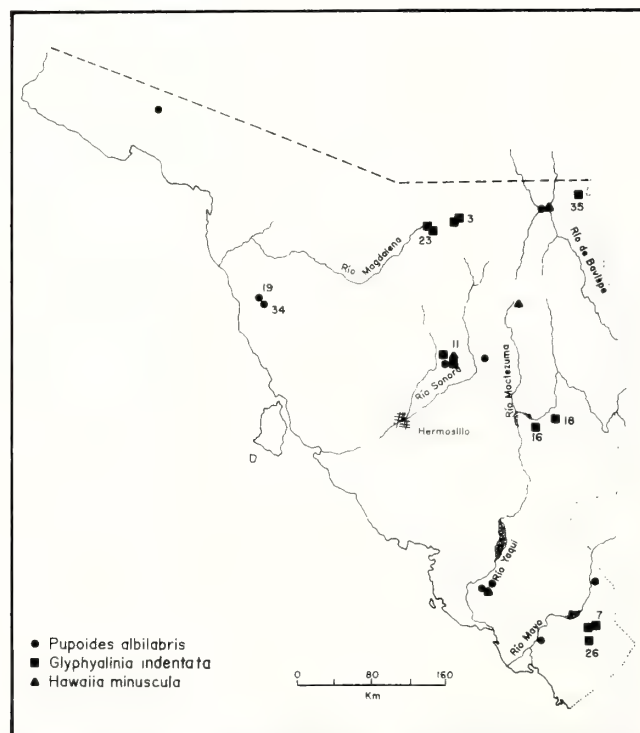


Fig. 2. Distribution of *Pupoides albilabris*, *Glyphyalinia indentata*, and *Hawaii minuscula* in Sonora.

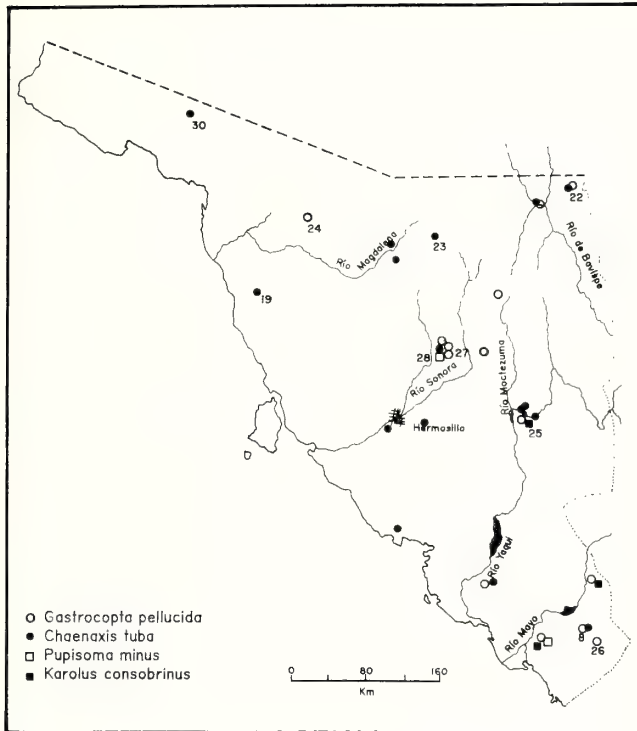


Fig. 3. Distribution of *Gastrocopta pellucida*, *Chaenaxis tuba*, *Pupisoma minus*, and *Karolus consobrinus primus* in Sonora.

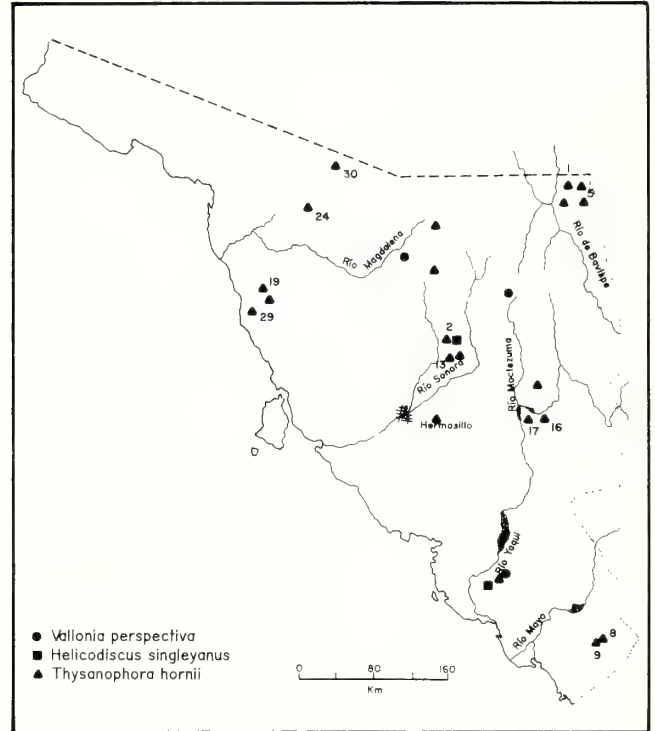


Fig. 5. Distribution of *Vallonia perspectiva*, *Helicodiscus singleyanus*, and *Thysanophora hornii* in Sonora.

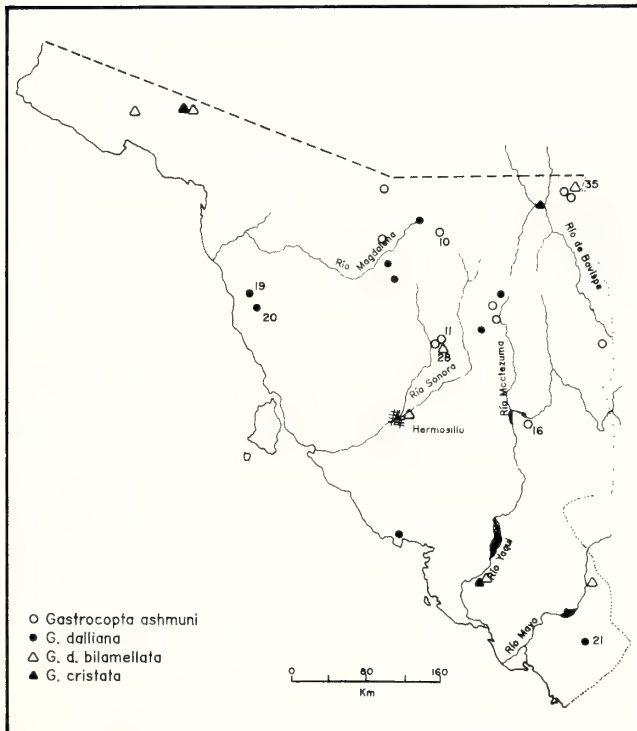


Fig. 4. Distribution of *Gastrocopta ashmuni*, *G. dalliana*, *G. d. bilamellata*, and *G. cristata* in Sonora.

of Arizona, New Mexico, trans-Pecos Texas, Chihuahua and Sonora; it is known from northern Sonora.

Gastrocopta l. dalliana dalliana (Sterki, 1898) (Fig. 4) is broadly distributed in east-central Arizona, also NW Chihuahua and Baja California Sur (Bequaert and Miller, 1973). It is apparently widely distributed throughout Sonora.

Gastrocopta l. dalliana bilamellata (Sterki and Clapp, 1909) (Fig. 4) occupies south-west and south-central Arizona. It is considered as representative of the very arid part of SW Arizona and NW Sonora (Bequaert and Miller, 1973). Its distribution seems to be over the west of Sonora with two records to the east. *G. d. dalliana* and *G. d. bilamellata* have not been found to co-occur in the state. Further collections are needed in order to obtain a better understanding of these subspecies ranges. Bequaert and Miller (1973) showed that the two subspecies overlap in central Arizona and suggested interbreeding among populations.

Gastrocopta (Immersidens) cochisensis (Pilsbry and Ferriss, 1910) (Fig. 1) is known from a single locality in Sonora (Pilsbry, 1953). With a broad distribution in southeastern Arizona (Bequaert and Miller, 1973), it has been recorded also from Animas Peak, Hidalgo County, New Mexico (Metcalf, 1976).

Gastrocopta (Gastrocopta) pellucida (Pfeiffer, 1841) (Fig. 3) is widespread in Texas and Florida, and scattered throughout the southeastern United States (Hubricht, 1985). It occupies SW United States from Texas to SE California, as well as Mexico and Central America south to Panama, and the Antilles (Bequaert and Miller, 1973). Those authors sug-

gested that *Gastrocopta pellucida* is a neotropical native that advanced from the south, and Mexico could be its center of dispersal.

Gastrocopta (*G.*) *cristata* (Pilsbry and Vanatta, 1900) (Fig. 4) is found mainly in central and western Texas, but ranges from Oklahoma to Arizona (Hubricht, 1985). It is common in southern Arizona, but is also known from Nebraska, Kansas and New Mexico (Bequaert and Miller, 1973). Its widely separated localities suggest an extensive range in Sonora.

There is one record of *Gastrocopta* (*Albinula*) *contracta* (Say, 1822) (Fig. 1), for Sonora (Pilsbry, 1953). It is widely distributed in the eastern United States (Hubricht, 1985) and also occurs in western, central, eastern and north-eastern Mexico (Bequaert and Miller, 1973).

Gastrocopta (*Vertigopsis*) *pilsbryana* (Sterki, 1890) (Fig. 1) is known from a single record for the state (Branson *et al.*, 1964). Pilsbry (1948) considered it one of the most common species of the highlands of New Mexico and Arizona. It also occurs in southern Utah, trans-Pecos Texas, and northern Mexico (Bequaert and Miller, 1973).

Chaenaxis tuba (Pilsbry and Ferriss, 1906) (Fig. 3) is broadly distributed in Sonora. The range of *Chaenaxis tuba* as shown by Bequaert and Miller (1973) is extended by several kilometers; to the west (present in the south and north ends of Sierra El Viejo range), to the south close to Sierra Alamos, and to the east near the border of Chihuahua state (found at Cerro Prieto). Bequaert and Miller (1973) regard this species as one of the most distinctive snails of the southwestern arid fauna.

Pupisoma minus Pilsbry, 1920 (Fig. 3) is a synonym of *P. macneilli* (Clapp, 1918) according to Hubricht (1985). *P. macneilli* occurs mainly in Florida and the southeastern United States. The species has been identified by shell material only (Pilsbry, 1953, this account), and for that reason I have decided to continue using *P. minus* for Sonora until live material becomes available for further studies.

Pupoides (*Pupoides*) *albilabris* (Adams, 1841) (Fig. 2) is widely distributed in the eastern United States (Hubricht, 1985), in South and North Dakota, Utah and Arizona (Bequaert and Miller, 1973). In Sonora a scattered distribution suggests that it is widespread throughout the state.

Vertigo (*Vertigo*) *ovata* (Say, 1822) (Fig. 1) is widely distributed in the eastern United States, rare in Arizona, and perhaps also in Sonora. There is one record for the state (Bequaert and Miller, 1973).

FAMILY VALLONIDAE

Vallonia perspectiva Sterki, 1893 (Fig. 5) has a dispersed distribution in eastern United States (Hubricht, 1985). It has an extended distribution in the highlands of Arizona, New Mexico and trans-Pecos Texas (Bequaert and Miller, 1973). The scattered localities of the species in Sonora suggest a large latitudinal range.

FAMILY FERUSSACIIDAE

Karolus consobrinus primus De Folin, 1870 (Fig. 3) is a snail mainly from eastern Mexico; for example Veracruz, from alluvial deposits of Rio Antigua (von Martens 1890-1901), Tampico, Tamaulipas from river debris (Pilsbry, 1907), Veracruz

Table 1. Species found in Sonora and localities. Numbers represent my localities (see Appendix). Asterisks indicate taxa shared with Arizona.

SPECIES	LOCALITIES	
FAMILY PUPILLIDAE		
<i>Gastrocopta</i> (<i>Immersidens</i>) <i>ashmuni</i>	5, 10, 11, 16, 28, 35	*
<i>G. (I.) dalliana</i>	10, 19, 20, 21, 34	*
<i>G. (I.) d. bilamellata</i>	28, 35	*
<i>G. (I.) cochisensis</i>		*
<i>G. (Gastrocopta) pellucida</i>	8, 11, 15, 22, 24, 25, 26, 27, 35	*
<i>G. (G.) cristata</i>		*
<i>G. (Albinula) contracta</i>		*
<i>G. (Vertigopsis) pilsbryana</i>		*
<i>Chaenaxis tuba</i>	16, 17, 19, 21, 22, 23, 27, 33	*
<i>Pupisoma minus</i>	28	
<i>Pupoides albilabris</i>	19, 27, 34	*
<i>Vertigo</i> (<i>Vertigo</i>) <i>ovata</i>		*
FAMILY VALLONIIDAE		
<i>Vallonia perspectiva</i>		*
FAMILY FERUSSACIIDAE		
<i>Karolus consobrinus primus</i>	32	
<i>Cecilioides</i> sp.	9	
FAMILY SUBULINIDAE		
<i>Lamellaxis</i> (<i>Allopeas</i>) <i>gracilis</i>		*
<i>L. sp.</i>	26	
FAMILY SPIRAXIDAE		
<i>Pseudosubulina</i> sp.		
FAMILY HELICODISCIDAE		
<i>Helicodiscus singleyanus</i>	28	*
<i>H. eigenmanni</i>		*
FAMILY VITRINIDAE		
<i>Glyphyalinia indentata</i>	3, 4, 5, 7, 8, 10, 11, 15, 16, 18, 23, 26, 28, 35, 36	*
<i>Hawaiiia minuscula</i>	11	*
FAMILY EUCONULIDAE		
<i>Euconulus fulvus</i>	26	*
FAMILY THYSANOPHORIDAE		
<i>Thysanophora hornii</i>	1, 2, 5, 6, 8, 9, 11, 12, 13, 14, 15, 16, 17, 19, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 35	*
<i>T. proxima</i>		

and Necaxa, Puebla (Baker, 1930). There are two records of the species for Sonora: Pilsbry (1953) and this account.

Cecilioides sp. (Fig. 1) was cited by Bequaert and Miller (1973) to be present in Sonora; however they did not specify a locality. I have a sample from Sierra Alamos, south Sonora. It is an element of a tropical family.

FAMILY SUBULINIDAE

Lamellaxis (*Allopeas*) *gracilis* (Hutton, 1834) was recorded for the state by Drake (1953:156) who mentioned that it was found "as far north as Hermosillo" without giving precise

localities. It is found in Arizona, and was introduced in the southern United States (Bequaert and Miller, 1973).

Lamellaxis sp. (Fig. 1) from southern Sonora differs from *Lamellaxis gracilis* by having widely spaced transverse ribs and a curved outer lip. The lip of *L. gracilis* is straight. Both show a high spire. This species is known from a single locality, Sierra Alamos.

FAMILY SPIRAXIDAE

Drake (1953) added *Pseudosubulina* to the species list for Sonora with a question mark, and no precise locality. The genus has been recorded in eastern, southeastern and southern Mexico (von Martens, 1890-1901).

FAMILY HELICODISCIDAE

Helicodiscus (*Helicodiscus*) *eigenmanni* Pilsbry, 1890 (Fig. 1) has once been recorded in the state by Pilsbry (1948). Bequaert and Miller (1973) considered it as a Southwestern snail, but it is also found in the south-central United States, and other scattered records exist for Colorado and South Dakota, Chihuahua and Puebla (central Mexico).

Helicodiscus (*Hebetodiscus*) *singleyanus* (Pilsbry, 1889) (Fig. 5) is found in several localities in the eastern United States (Hubricht, 1985), west to southeastern South Dakota, Colorado, Kansas, Oklahoma, and Texas. In Mexico there are records from Tamaulipas and Sonora (Bequaert and Miller, 1973). It has two records in Sonora which could suggest a widespread distribution.

FAMILY VITRINIDAE

Glyphyalinia indentata (Morelet, 1851) (Fig. 2) is widespread latitudinally in eastern Sonora. It is also broadly distributed in the central-eastern United States (Hubricht, 1985), west to Oklahoma, Texas, New Mexico, Utah, and Arizona, and from Mexico to southern Guatemala (Bequaert and Miller, 1973).

Hawailia minuscula (Binney, 1841) (Fig. 2) is extensively distributed in the eastern United States (Hubricht, 1985). It could be the commonest snail of Arizona. It is found from North America to Central America and the Antilles. Nowadays it is almost cosmopolitan due to introductions (Bequaert and Miller, 1973).

FAMILY EUCONULIDAE

Euconulus fulvus (Müller, 1774) (Fig. 1) was not known previously from Sonora. This account includes a single record from Sierra Alamos. It is a Holarctic snail, and it is found over most of the United States (Bequaert and Miller, 1973).

FAMILY THYSANOPHORIDAE

Thysanophora hornii (Gabb, 1866) (Fig. 5) is the commonest microgastropod of Sonora. It is also found in Arizona, New Mexico, and southern Texas (Hubricht, 1985). It is mainly known from the northern states of Mexico, to Sinaloa and Jalisco (Bequaert and Miller, 1973). These authors regarded *T. hornii* as an atypical Neotropical invader.

Thysanophora proxima Pilsbry, 1899 (Fig. 1) has one record for the state by Branson, *et al.* (1964). This seems to be the northeast locality since it was described from Uruapan, Michoacán (Pilsbry, 1899 and 1903) in west-central Mexico.

Pilsbry gave other localities in Michoacán state.

Thysanophora hornii is the most widely distributed species in the state, followed by *Glyphyalinia indentata*, *Gastrocopta pellucida* and *Chaenaxis tuba*.

It should be noted that several species, such as *Gastrocopta contracta*, *G. cochisensis*, *G. pilsbryana*, *Vertigo ovata*, *Thysanophora proxima*, and *Helicodiscus eigenmanni*, known from previous publications, as well as three species, *Cecilioides* sp., *Euconulus fulvus* and *Lamellaxis* sp., from my field samples, are known only from single records (Fig. 1).

Other specimens display an apparently spotty distribution, e.g. *Gastrocopta cristata* (Fig. 2), *Vallonia perspectiva* (Fig. 5), *Helicodiscus* (*Hebetodiscus*) *singleyanus* (Fig. 5), and *Pupisoma minus* (Fig. 3). Nevertheless, the records are so scattered as to suggest a possibly wider distribution.

The present distribution of *Gastrocopta* (*Immersidens*) *ashmuni* (Fig. 4) indicates an extension of the Southwestern Molluscan Province to the south, while *Karolus consobrinus primus* (Fig. 3), indicates an extension of a Mid-western Mexican Province to the north. The available information does not yet allow the identification of a biotic community with particular significance for the distribution of microsnails in Sonora.

The new localities for the Sonoran fauna represent an additional contribution to the regional molluscan biogeography as discussed by Bequaert and Miller (1973), by supporting the Southwestern Molluscan Province suggested by Henderson (1931), and providing evidence that it extends farther south than Henderson indicated. The records of snails from the southern part of Sonora clearly represent populations with affinities to a biogeographical zone containing tropical components, such as species of the families Ferussaciidae, Subulinidae and perhaps Spiraxidae.

The micromolluscan fauna of Sonora shows a strong affinity with the Southwestern Molluscan Province of Henderson (1931), with an additional weak introduction from the south, from what could be called a Mid-western Mexican Province to be better understood when more information becomes available. More field work needs to be done in the state in order to reach a more comprehensive conclusion.

ACKNOWLEDGMENTS

I wish to thank Dr. Walter B. Miller, James Hoffman, Ronnie Sidner and Diana Warr for their companionship in the field, and Teresa Olivera and Oscar J. Polaco from the Laboratorio de Prehistoria, Instituto Nacional de Antropología e Historia for allowing me to utilize their installations in the identification of some material. Ricardo Ayala, Tim A. Pearce, and two anonymous reviewers gave various ideas.

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Date of manuscript acceptance: 17 April 1990

Appendix

KEY OF LOCALITIES:

1. Cerro Gallardo, ca. 1.5 km due S of Rancho Gallardo buildings, in north-facing rhyolite rockpiles, 31° 17.8' N, 109° 23.4' W, elev. 1500 m.
2. At Rancho Seco, along Carbo - Rayon road. 29° 41' N, 110° 40.1' W.
3. Along road to Microondas installations, Sierra Mariquita ca. 40 km W Cananea at jct road - Hwy 2, 31° 03.5' N, 110° 22.5' W, elev. ca. 2000 m.
4. El Quince, ca. 7.8 miles by road W Cananea at El Quince, Sierra Elenita, 29° 40.1' N, 110° 47.5' W.
5. Sierra Las Minitas, ca. 2 km due SE of Rancho El Jucaral buildings; in rhyolite rockslide, 31° 11.1' N, 109° 04.7' W, elev. ca. 1400 m.
6. 5 miles South of Cucurpe, along Rio San Miguel Horcacitas, on N facing slope, 30° 15' N, 110° 43.5' W, elev. ca. 830 m.
7. Sierra San Ignacio, ca. 2 km E from El Naranjo, 27° 14' 51.8'' N, 108° 45' 38'' W.
8. Sierra San Ignacio ca. 1 km E Rancho Agua Salada, 27° 15' 03'' N, 108° 46' 15'' W, elev. ca. 600 m.
9. Sierra Alamos, Arroyo Las Piedras, S Alamos. 26° 59' 22'' N, 108° 57' 22'' W.
10. At S edge of Sierra El Pinito ca. 10.1 km W Aribabi on road Imuris-Cananea (Hwy 2), N facing limestone slide. 30° 52.7' N, 110° 42.7' W.
11. High, N facing rockslide on slope above arroyo, along Rayon-Cerro de Oro road, at ca. 4.2 miles from Cerro de Oro. 29° 38.9' N, 110° 36.6' W.
12. Ravine along road from Rayon to Cerro de Oro, in small rockpile at base of left bank of arroyo ca. 2.6 mi from Cerro de Oro. 29° 38.3' N, 110° 37.7' W.
13. Mina San Jose (Zona de Minas) along dirt road from Rayon to Cerro de Oro. 29° 37' 17'' N, 110° 37' 48.8'' W.
14. Rancho La Tarasca (Rancho Las Rastritas) on banks of Rio Los Lobos. 29° 37.5' N, 110° 38.5' W.
15. Behind hill of arroyo along Rayon-Cerro de Oro road, ca. 4.2 mi from Cerro de Oro. 29° 39.6' N, 110° 35.3' W.
16. Sierra Batamote, along left side of La Estrella - Sahuaripa road, on hill facing Rancho El Torreoncito. Mina El Milagro. 28° 57' 09.7'' N, 109° 32' W.
17. Cerro La Mona, on left side of road from Hermosillo to Mazatan, ca. 21 km E Hermosillo. 29° 02' 54.4'' N, 110° 39' 22.5'' W.
18. At left side of road, on road from Sahuaripa to Hermosillo, ca. 29° 01.5' N, 109° 18.7' W.
19. Sierra El Viejo, north end of range, on N-facing limestone piles, 30° 24.1' N, 112° 22.5' W, elev. ca. 550 m.
20. Sierra El Viejo, SW part of range, along W running wash, on granite rockpiles, 30° 18.7' N, 112° 20' W, elev. ca. 540 m.
21. Rancho Tres Marias, W of Alamos, ca. 1 km on road to Conicarit from junction with Hwy 1 (Alamos-Navojoa). 27° 06' 56'' N, 109° 09' 18'' W.
22. Cerro Prieto, ca. 1.5 km west Ejido 18 de Agosto on basalt rocks, ca. 31° 14.5' N, 109° 16.2' W.
23. At S edge of Sierra El Pinito, ca. 12.8 km W Aribabi, along Imuris - Cananea road (Hwy 2) on W facing limestone rockslide, 30° 52' 20'' N, 110° 43' 10'' W.
24. Low and small range along right side of road from Altar to Sasabe. 30° 58.7' N, 111° 45.4' W.
25. On a small hill in front of La Estrella (near El Novillo) on left side of road from Hermosillo to Sahuaripa, ca. 28° 56.8' N, 109° 38.5' W.
26. At ESE part of Sierra Alamos, (Mina abandonada) Arroyo Las Piedras, 26° 59' 37.5'' N, 108° 56' 36.4'' W.
27. Arroyo Cerro de Oro, ca. ¼ mile from Cerro de Oro on road to Rayon. ca. 29° 36.8' N, 110° 37.8' W, elev. ca. 600 m.
28. Cerro de Oro (WBM) sample. Arroyo Cerro de Oro, ca. ¼ mile from Cerro de Oro on road to Rayon. ca. 29° 36.8' N, 110° 37.8' W, elev. ca. 600 m.
29. NW end of Sierra Pico, in igneous rocks at base of cliffs, along road from El Plomito to Puerto Libertad, at 23.8 km from El Plomito; ca. 365 m. ca. 30° 04' 16.2'' N, 112° 25' 18.2'' W.
30. Sierra Pozo Verde, Cerro El Sasabe, NE slope, ca. 4 km S of Sasabe, along road to Altar, 31° 26.3' N, 111° 33.8' W, elev. ca. 1050 m.
31. Arroyo Los Alamos, E side Rio Yaqui, ca. 1.5 km S El Novillo, 28° 58.1' N, 109° 37.5' W, elev. ca. 260 m.
32. SW part of Sierra Batamote. On ravine along La Estrella-Sahuaripa road few miles from La Estrella, ca. 27° 57' N, 109° 32' W.
33. Sierra El Viejo SW part of range, along W running wash, on a limestone rockslide, 30° 18.7' N, 112° 20.2' W, elev. ca. 540 m.
34. Sierra El Viejo N end of range on granite rockpiles, 30° 23.8' N, 112° 23.6' W, elev. ca. 500 m.
35. SW end of Sierra Los Embudos, *circa* ½ km E of Rancho El Jucaral. 31° 11.8' N, 109° 05.2' W.
36. Sierra Mariquita, along road to Microondas installations, ca. 40 km W Cananea at jct road - Hwy 2, 31° 02' 22'' N, 110° 22' 25'' W.

SENTENTĪA

THE NEXT CHALLENGE: LIFE STYLES AND EVOLUTION

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ABSTRACT

Knowledge of Western North American land snails, especially those inhabiting desert or semi-arid regions, still rests in the exploratory-descriptive stage. Technological advances in transportation have shifted travel from the "hiking and horseback" two month camping trips made early in this century by Pilsbry and Ferriss, to the current 4-wheel drive vehicles used in weekend dashes. Inevitably technology will shift to other modes of transport — but the dust and sweat of rock moving will remain.

We have found more species, and accumulated data on what lives in additional mountain ranges, but we still know almost nothing of these snail's life histories. Generalizations concerning their patterns of variation, ecology, and structure must be abstracted from the early descriptive-faunistic papers of Pilsbry and Ferriss (1905, 1910, 1915) or the seminal report by Gregg (1960) on genital reductions in true desert taxa. These snails do not have a "natural history" elucidated. Without these data, attempts to interpret their evolution and biogeography will be simple academic exercises.

A brief commentary on Australian arid zone taxa suggests many avenues for North American investigations, and that the current "hyperinflation" of suprageneric taxonomic units probably results from our ignorance of desert snail biology and evolution.

There are a happy few of us who have experienced the joys, frustration, rewards, bruises, thrill of discovery, and comradeship of collecting land snails in the deserts of the world (e.g. Ferriss, 1904). In Western North America, for nearly 30 years, this has been the basic provenance of Walter Miller and his succession of graduate students, here christened "Miller's Mob".

At the same time, there are those who "put up with" the chosen few. A significant gap in this symposium is the failure to permit Walt's gracious wife a few words on being the "wife of a snailer". This subject has been reviewed delightfully by Frances Norman Young (1954) in her "A Word From The Wife Of A Tree Snail Hunter", referring to her husband's "obsession" with Florida Tree Snails of the genus *Liguus*. In her husband's words, "...snail hunting combines all the pleasures of an Easter Egg Hunt with the dangers and thrills of exploration in tropical jungles" (Young, 1954: 151). All of us desert rats who have backed into a cactus while

stooping to pick up a shell, trod on a rattlesnake, grabbed a centipede or large scorpion while turning a rock, can testify that desert joys are not second to those of the Everglades.

And it is the thrill of *exploration* that at least partly guides current work. Pick an isolated and unvisited mountain range or two, drive to the foothills, hike in, and demolish a few rock piles to extract some "live snails". A part of this process is to verbally share your efforts by shouting messages to people on the next hillside, using the arcane language of "snailer's progress" as the rock pile is changed to an "open pit". First, there are the near surface "bones" — weather-beaten long dead shells; then "fresh shells" — snails dead only a year or two; excitement and assurance of success mounts at the first sighting of the characteristic coils of dried excrement that prove living snails are nearby; "juveniles" — first *live* examples discovered; and finally the triumphant call of "live adult".

Although not part of "Miller's Mob", I did share some weekend camps with Walt Miller, "Doc" Gregg, and Munroe Walton. This was in the early 1960's, when I cut my teeth on

¹Published posthumously with minor editorial revision.

desert snails. But my interests turned to the Pacific Basin, and then to the semi-arid areas of Australia.

Back in the laboratory, the snails were fixed and preserved, genitalia dissected out, cleared and prepared for study. "Miller's Mob" followed the technique developed by "Doc" Gregg and perfected by Walt Miller, of making permanent slide mounts of stained genitalia. Differences are noted from previously known species, illustrations prepared, and publications extracted from longer theses. Gradually our knowledge of the land snail distribution patterns grew and the number of described species increased.

But surprisingly little new data were added concerning life history, evolution, or biogeography. A dedicated amateur, Munroe L. Walton, whose ambition was to collect every species of land snail in the Western United States from its type locality, kept a few live snails in his basement, eventually permitting a series of notes on longevity of different genera (Walton, 1963, 1970). And "Doc" Gregg (1960) published an abstract of a major work tracing the evolution of desert helicoid genera by reduction in genital structures.

It remains necessary to use the reports of Pilsbry and Ferriss (1905: 226-227; 1910: 47-53; 1915: 363-364) for generalizations as to ecology and variation patterns among the species. They pointed out that "Our work is a reconnaissance rather than a complete survey" (Pilsbry and Ferriss, 1915: 363). The many added finds made by Walt Miller and his students confirm this view, and even in the "best explored ranges", such simple facts as the total range of any species remain a mystery.

THE MODERN VIEW

As is evidenced by the contributions of this symposium, malacologists have been exposed to modern ideas concerning cladistics, continental drift and "Pacifica", phyletics, and vicariant biogeography. Undoubtedly land snail data will have much to contribute to discussions of these subjects. But our understanding of the significance and evolutionary origin of observed structural differences is very poor. Without such understanding, interpretation of differences will be on the most basic phenetic level, and hence of little import.

We see differences in genital structures among species of the many genera, and assume that these indicate that speciation has occurred. We see similarities in structure among taxa from different ranges, but are these convergent adaptations to similar environmental parameters? Or are they evidence for speciation by vicariation?

We cannot answer such simple questions as: How long do the species live? How long before they become sexually mature? Are there seasonal variations in genital structure? When do they reproduce? Are mating and egg laying in the same "activation", or separated by a period in diapause? What percentage of the year are they active in different vegetation zones and ranges? How many times during a year are they activated? What do they feed on?

Especially under the stress of desert conditions, when the number and length of activations will be minimal, are there competitive interactions among sympatric species or not?

Has the reproductive cycle of species shifted from that found in areas with a "dependable" annual period of rain to a different pattern in places where rain comes only occasionally and unpredictably? Are there structural changes that can be correlated with increased aridity? Did the snails colonize the arid areas? Or did increased aridity come to the snails?

For many of the arid zone land snails in Western North America, the currently available data on genital structures, ranges, and climatic parameters could enable initial estimates of environmental correlation and patterns of changes. But much basic life history observation will need to be accomplished before the full evolution of these fascinating taxa can be understood.

TRANSCONTINENTAL COMPARISONS

For the past 15 years I have been monographing the camaenid land snails from the western two-thirds of Australia, and puzzling over their patterns of structural and ecological specializations. The basic monographs are now completed, identifying 356 species (234 new) in 51 genera (24 new), with many more species to be discovered.

From this massive data set, a number of patterns have emerged, usually correlating closely with moisture regimes. Where there is a dependable and predictable annual period of rain, maturation takes four years, there are considerable seasonal shifts in the size of individual reproductive organs (reduced in size during late wet season and through most of the dry season in order to maximize food storage), little or no evidence of feeding specializations, and generally very large genitalia. Where the annual rainfall is greatly reduced, but still dependable and predictable, patterns of maturation and seasonality remain, but feeding specialization become common, and both the size and complexity of the genitalia tends to be reduced. In areas where the rainfall is sporadic, unpredictable, and usually slight (relieved by rare torrential drenchings), the snails show major feeding shifts, must be ready to reproduce ANY time it rains (hence seasonal shrinkage of reproductive organs is no longer possible), the need for increased food storage has resulted in functional shifts among organs, and there has been drastic decrease in general body size and terminal genital organs. These alterations decrease energy use and thus improve the chance of survival in marginal desert areas.

These changes are repetitive and recur in unrelated taxonomic lineages. I anticipate that equivalent patterns can be discovered in the New World taxa, and believe that the hunt for and probable recognition of such patterns is a necessary prelude to sound phylogenetic models.

After a lengthy period of neglect, malacologists are suddenly paying attention to family level classifications and problems, [see for instance Schileyko (1989, abstract)]. Only a decade ago, Solem (1978: 93) listed but one superfamily and three families in the Helicacea. Schileyko (this symposium) now splits the old Helicacea into three superfamilies, 11 families, 33 subfamilies, and 18 tribes - after subtracting a few groups. Such "malacological hyperinflation" hopefully

will stop short of the example set by ornithologists, where about 12,500 *generic* names are available for only 9,000 *species* of birds! The amount of analytic work that has gone into the revisions of Schileyko and Nordsieck is significant, but I think they are flawed by a failure to appreciate the plasticity and adaptability of the molluscan body in adjusting to living conditions in semi-arid and desert area. Plus the fact that these changes have happened many times.

It is clear that much remains to be done before we can hope to understand the phylogeny of land snails and establish an adequate phylogenetic classification. My own view is that the many semi-arid and desert faunas will provide many problems and special opportunities for study, and that loss or size reduction of structures has been a fundamental and repeated evolutionary sequence. Until this is recognized, patterns analyzed, and the consequences of such convergences allowed for in phylogenetic reconstructions, the proposed classifications will be less than robust.

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Date of manuscript acceptance: 21 July 1990

BOOK REVIEW

Squid as Experimental Animals

Gilbert, D. L., W. J. Adelman, Jr., and J. M. Arnold, eds. Plenum, New York, 1990. 516 pp. \$75.00.

It is nice to see a new book about cephalopods because until recently there were precious few of them. *Squid as Experimental Animals* weighs in at just over 1½ lbs., with over 500 pages, 22 chapters, 34 contributors and no fewer than 3 editors. It is a successor to a modest handbook of 74 pages with 6 authors, published in 1974, and entitled *A Guide to the Laboratory Use of the Squid Loligo pealei*, and its size reflects both the advantages and disadvantages of having more knowledge. Of course, it is a great tribute to squids that many biologists devote at least their summers to studying them and the idea of bringing together the work of North American squid experts in a single volume like this obviously has much to commend it. The editors are to be congratulated on their enterprise and the book will undoubtedly be very useful. However, expansion of a field should not automatically mean expansion of all treatments of it, and in my view, this guide book would have been immeasurably improved if it had been kept shorter.

There are six sections. The first, Evolution, History and Maintenance, comprises 58 pages and is, as its name suggests, a hotch potch. A lengthy chapter on rearing and culturing squids (Hanlon) is the meat here and there is also a little natural history, though alas without any pictures. It also includes two bizarre indulgences, in one of which the evolution and intelligence of cephalopods are despatched in a mere half a dozen pages. The second section, Mating Behaviour and Embryology (25 pages long), comprises two chapters by the same author (Arnold) so that there is uniformity here and some useful data are clearly presented. I was sorry, however, to see that the only pictures in the entire book of squids behaving showed such strange, grey-looking creatures. For I do not believe that *Loligo pealei* maintains such a uniform appearance during courtship and copulation and indeed the pictures belie some of the author's own description.

The third and fourth sections (99 pp. and 172 pp., respectively) constitute the bulk of the book. They are broadly devoted to what has come to be known as "axonology", though the editors divide the ten chapters somewhat arbitrarily into *Neural Membranes* (Section III) and *Cell Biology* (Section IV). The treatment here is very uneven indeed. First in length: in the shortest chapter, the cut-open axon technique is described in only seven pages yet the authors of the cytoskeleton chapter are allowed to run to 68 printed pages! The median length is 20 pages but the fact that the chapter by two of the editors (Adelman and Gilbert) is 40 pages long seems evidence that they do not rate brevity particularly high and so were hardly likely to demand it from their contributors. More seriously, the treatment is also uneven, more so, in fact, than is usually associated with a multi-author work: were all the authors really given the same brief by the editors? If they

were and some authors ignored it then the editors should have called the transgressors to order. But Adelman and Gilbert themselves (Ch. 7) present such an uneasy mix of history, tabulated data and banal figures that we can hardly expect much better of the other authors.

Some of the chapters in this part of the book will undoubtedly be very useful for beginners in the field and we may mention here the chapters by Bezanilla and Vandenberg (Ch. 9), Stanley (Ch. 11), Rice, Mueller and Adelman (Ch. 12), R. S. Cohen, Pant and Gainer (Ch. 13), and Weiss, Meyer and Langford (Ch. 15). On the other hand some will disappoint: the chapter on the potentially fascinating axoplasm by Brown and Lasek (Ch. 14) is so long that this reviewer at least had great difficulty in seeing the wood for the trees. How I wish the editors had been tougher and bullied the authors into telling us just what it was they had found out. In fact this brings me to my chief concern with this part of the book: what exactly is being found out these days about the axon? For example, internal dialysis (Ch. 8) and optical measurements (Ch. 10) are all very well but I could not discover what exactly they have added to our understanding of the axon. Indeed Gould and Alberghina (Ch. 16) frankly admit that the function of most lipids in the axon is "a mystery", although they only come clean after 46 pages! We all knew that J. Z. Young, Cole, Curtis, Hodgkin, Huxley and Baker were "giants" but, my goodness, they really do seem to have creamed off the very best bits, didn't they? Just where is "axonology" going?

The fifth section, Sensory Systems, which comprises 67 pages, offers the reader the best value for money in the volume. Saibil and Meinertzhagen on the eye (Chs. 17 and 18) and Budelmann on the statocysts (Ch. 19) produce no verbiage, and, as well as providing admirable summaries these chapters offer the kind of practical technical information a potential reader of this book might need, often moreover in an easily accessible form. And they summarise important and elegant work.

The last section (Part VI), Integrated Systems, is another hotch potch, a short account of an organophosphorus detoxifying enzyme nestling cheek by jowl with an interesting account of gas transport (Mangum) and a nice chapter on physiological performance (O'Dor, *et al.*), which clearly is about what I take to be an "integrated system", i.e. an animal.

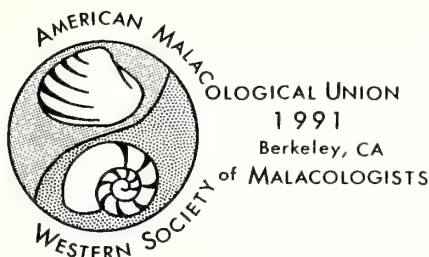
There is no overview or summary; but there is a subject index. References are wisely left attached to their appropriate chapters. So overall the book is like the curate's egg, excellent in parts. Whether it could be said to meet the Editors' aim of being "an introduction to squid biology" is a moot point, however, and one that readers of the *American Malacological Bulletin* will need to ponder. Obviously it will be very useful for graduate students and for workers experienced with other

animals coming new to *Loligo*. I have already drawn attention to the sort of chapter I would expect to be really useful to such people: Saibil's chapter on the eye would be my model. But what a pity the editors did not include an introductory chapter with a few diagrams to show the beginner the main external features of *Loligo* and the major organ systems - a re-working of L. W. Williams (1909) in fact.

However I do not want to go on about the editors. Lots of the authors are to blame for prolixity and even more importantly for a feature that as a Zoologist I found especially disappointing. The biology of these wonderful animals is

somehow missing in this book: it is as if all the boring bits got put in and the exciting parts left out! Those of us who are fortunate enough to have worked with living squid or the ravishingly beautiful tissues that can be dissected out of freshly killed squid will not be able to use this text to try to convey to our less fortunate students and colleagues the sheer beauty and excitement of many of the systems that the contributors to this volume discuss.

—John Messenger, Dept. of Zoology
University of Sheffield
Sheffield 10, England



**57TH ANNUAL MEETING
THE AMERICAN MALACOLOGICAL UNION
BERKELEY, CALIFORNIA
JUNE 30 - JULY 5, 1991**

The 57th annual meeting of the American Malacological Union will be a combined meeting with the Western Society of Malacologists, held June 30 - July 5, 1991 at the Clark Kerr Campus of the University of California, Berkeley. The conference center provides a comfortable and convenient complex of meeting facilities, adjacent dining facilities, and guest accommodations in a Spanish style complex separated by lawns, enclosed gardens and courtyards. The Campus is nestled into 43 acres overlooking San Francisco Bay and stretching upward into the Berkeley Hills.

The conference center will offer a special package of meals and accommodations, in a combination of residence halls and suites. A variety of hotel and motel housing will be available to those who wish to stay off campus. The Bay Area is filled with diverse cultural and natural history attractions as well as recreational and sight-seeing opportunities. It is famous for its restaurants and fine cuisines. Berkeley summers are free of rain and are regularly cooled (sometimes chilled) by the renowned San Francisco Fog.

Three symposia are planned:

**MARINE BIVALVE RESEARCH IN THE NEXT CENTURY,
A REVIEW OF THE CURRENT STATE OF OUR KNOWLEDGE AND DIRECTIONS FOR THE FUTURE**
(Organized by Drs. Paul H. Scott, Brian Morton, and Eugene V. Coan)

MOLLUSCAN TAPHONOMY AND PALEOECOLOGY
(Organized by Drs. Carole S. Hickman and Michael P. Russell)

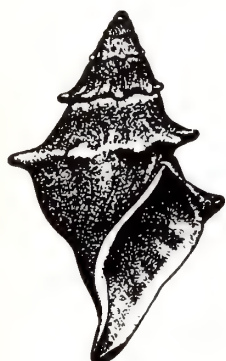
MOLLUSCAN BIOGEOGRAPHY OF THE PACIFIC BASIN
(Organized by Drs. David R. Lindberg and Geerat J. Vermeij)

In addition to the symposia, contributed papers, and poster presentations, scheduled events will include field trips, an outdoor barbecue, a joint AMU/WSM auction, a dessert reception at the Museum of Paleontology, and a 4th of July Banquet in the Great Hall of the Faculty Club. Fossil and Recent mollusk collections in the University of California Museum of Paleontology and the California Academy of Sciences in San Francisco will be available to meeting participants before, during, and after the meeting.

For further information please contact:

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The Special Publication Series of the *American Malacological Bulletin* was begun to disseminate collected sets of papers with similar or related themes in a single volume. To date, three such issues have been published, each the result of a special convened symposium. The three Special Editions are *PERSPECTIVES IN MALACOLOGY*, *PROCEEDINGS OF THE SECOND INTERNATIONAL CORBICULA SYMPOSIUM*, and *PROCEEDINGS OF THE SYMPOSIUM ON ENTRAINMENT OF LARVAL OYSTERS*. Additional Special Editions are planned for the near future.

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Dates of Publication

Volume 8(1), August, 1990

Volume 8(2), April, 1991

CONTRIBUTOR INFORMATION

The *American Malacological Bulletin* serves as an outlet for reporting notable contributions in malacological research. Manuscripts concerning any aspect of original, unpublished research, important short reports, and detailed reviews dealing with molluscs will be considered for publication.

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All binomens should include the author attributed to that taxon the first time the name appears in the manuscript [e.g. *Crassostrea virginica* (Gmelin)]. This includes non-molluscan taxa. The full generic name along with specific epithet should be written out the first time that taxon is referred to in each paragraph. The generic name can be abbreviated in the remainder of the paragraph as follows: *C. virginica*.

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Seed, R. 1980. Shell growth and form in the Bivalvia. In: *Skeletal Growth of Aquatic Organisms*, D.C. Rhoads and R.A. Lutz, eds. pp. 23-67. Plenum Press, New York.

Vail, V.A. 1977. Comparative reproductive anatomy of 3 viviparid gastropods. *Malacologia* 16(2):519-540.

Yonge, C.M. and T.E. Thompson. 1976. *Living Marine Molluscs*. William Collins Son & Co., Ltd., London. 288 pp.

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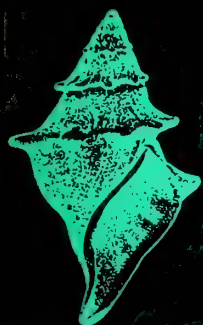
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AMERICAN MALACOLOGICAL BULLETIN

VOLUME 9

NUMBER 1

Biannual Journal of the American Malacological Union

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THE AMERICAN MALACOLOGICAL BULLETIN is the official journal publication of the American Malacological Union.

AMER. MALAC. BULL. 9(1)

ISSN 0740-2783

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EDITORIAL COMMENTS

The *American Malacological Bulletin* has strived over the years to offer the malacological community a diverse and high quality series of articles on molluscan biology. Since its inception in 1983, the *AMB* has undergone an evolution leading to the package you now hold in your hands. To celebrate the changes and evolution of the *Bulletin*, as well as the continued success of the American Malacological Union, we have altered our journal appearance. As you can note, the articles are in a different format, one that we hope satisfies both the professional and aesthetic tastes of our readers. The publication date of a particular issue will no longer be given in that issue, because it can not always be guaranteed that this date represents the exact date of distribution (see ICZN Code, Art. 21). The publication date of a current issue will therefore be published in a later issue. Additionally, to denote these changes, there are alterations in our contributor information that clarify and highlight expectations of our journal and the desire to increase the level of submissions dealing with new molluscan taxa. The new cover stock also reflects these changes.

We continue to hope our readership and members of AMU find the *American Malacological Bulletin* an important contribution to malacology. We appreciate the support of our Reviewers, Editorial Board, AMU Members and Council and general readership.

RSP, Editor-in-Chief



Observations on the anatomy of the scaphopod mantle and the description of a new family, the Fustiariidae

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Abstract. The mantle of five genera of the order Dentaliida and ten genera of the order Gadilida are investigated using histological and ultrastructural methods. The anterior mantle edge is divided into three functional units: 1) a frontal epithelium; 2) a central fold; 3) an inner gland region. In the Dentaliida the frontal epithelium is differentiated as an outer gland region and the central fold carries an annular ciliary organ. *Rhabdus rectius* (Carpenter) (Dentaliida) has a pair of ciliated slits instead of the annular organ. Neither feature is found in the Gadilida, where the frontal epithelium forms sensory papillae. Different types of ciliary receptors in the anterior mantle edge of both orders are present. The inner gland region in the Dentaliida consists of epithelial gland cells only; in the Gadilida both epithelial and subepithelial gland cells are present. The epidermis of the pallial cavity is low cuboidal but features ciliary rings in the anal region.

The posterior pallial edge or pavillon of the gadilids has a powerful ciliary organ to produce water currents and ciliated ridges on the dorsal mantle process; both structures are lacking in the dentaliids. *Fustiaria* differs from other genera of the Dentaliida by lacking a ventral bolster of connective tissue at the posterior mantle margin. Abundance and distribution of subepithelial gland cells vary between genera. The validity of the family Rhabdidae is supported and a new family, the Fustiariidae, is proposed.

The anatomy of the Scaphopoda has received the least attention of all of the classes of the molluscan subphylum Conchifera. Early accounts, e.g. Deshayes, 1825; Lacaze-Duthiers, 1856-7; Fol, 1889; Plate, 1892; and Simroth, 1894, however, contained excellent studies of scaphopod morphology and histology that remain valid in many aspects. Although almost all investigations were made on a single genus, *Dentalium* (= *Antalis*) (order Dentaliida), the results have been generalized subsequently for all Scaphopoda.

Lacaze-Duthiers (1856-7), Plate (1892), and Boissevain (1904) described the histological structure of the mantle in *Antalis entalis* (L.). They noted an outer glandular region at the anterior mantle edge, including two to three types of gland cells, a ring of connective tissue supporting the mantle opening, and an inner glandular region consisting of mucus-producing cells. Plate (1892) found that the genus *Cadulus* lacked the outer gland region, and later Odhner (1931) confirmed. Boissevain (1904) reported a ring of ciliated cells between the gland regions that she considered to be a sensory organ. Gabe and Prenant (1950) included the anterior mantle edge of *A. entalis* in their histological studies of the scaphopod connective tissue.

In the midregion of the animal, ciliated ridges were found just anterior to the anal opening in *Antalis entalis* and *A. dentalis* (L.) by Lacaze-Duthiers (1856-7) and Fol (1889). Distaso (1906) described them in more detail and also claimed the presence of an osphradial sense organ. Leon (1895) and

Boissevain (1904) described the posterior mantle edge, the so-called pavillon, and reported a small ciliated area and scattered gland cells. Reynolds (1988) investigated sensory cells of the pavillon of *Rhabdus rectius* (Carpenter) (Dentaliida).

This paper presents a comparative anatomy of the mantle in selected members of the orders Dentaliida and Gadilida (= Siphonodentaliida) to demonstrate the anatomical diversity in Scaphopoda and to provide new information for a better founded classification.

MATERIALS AND METHODS

The species studied are listed in Table 1. Mediterranean species were collected by means of SCUBA or by a triangle dredge from 8-20 m in Rovinj and Piran (Peninsula of Istria, Yugoslavia). In Norway, animals were gathered by a "Mini-Sanders" epibenthic sledge and box-core sampler from 120-590 m in the fjords near Blomsterdalen, Bergen. The animals from Puget Sound, Washington, U.S.A., were collected with a triangle dredge from 75-100 m near Waldrun Island.

HISTOLOGICAL PREPARATION

Living animals were narcotized with isotonic MgCl₂ solution (1:2 to 1:1 with sea-water) or MS 222 added to the sea-water (1:4000 - 1:2000). Tissues were fixed in 2-4% formalin or 4% glutaraldehyde buffered in sea-water. Helly's

Table 1. Systematic list of the species examined (USNM, United States National Museum of Natural History, Smithsonian Institution; NMNZ, National Museum of New Zealand; ¹Museum National d'Histoire Naturelle, Paris; ²Gareth Davies, University of Edinburgh; ³Zoologisk Museum, Copenhagen; *prepared for TEM methods)

Order DENTALIIDA		
Family		Source
Dentaliidae		
<i>Dentalium laqueatum</i> Verrill, 1885		USNM 765278
<i>D. neohexagonum</i> Sharp and Pilsbry, 1897		Monterey Bay
<i>Graptacme calamus</i> (Dall, 1889)		USNM 801250
<i>Antalis dentalis</i> (L., 1767)		Rovinj*
<i>A. entalis</i> (L., 1758)		MBI ²
<i>A. inaequicostatum</i> (Dautzenberg, 1891)		Piran*
<i>A. occidentalis</i> (Stimpson, 1851)		Bergen, Norway*
<i>A. vulgaris</i> (Dacosta, 1778)		Rovinj
<i>A. sp.</i> BS 660		NMNZ BS660
<i>A. sp.</i> P 927 II		NMNZ P927 II
<i>A. sp.</i> 930		NMNZ P930
<i>A. sp.</i> Q 719		NMNZ Q719
<i>Fissidentalium candidum</i> (Jeffreys, 1877)		AT 186 ²
<i>F. majorinum</i> (Mabille and Rochebrune, 1889)		USNM 709081
<i>F. megathyris</i> (Dall, 1899)		Galathea St716 ³
<i>F. zelandicum</i> (Sowerby, 1860)		NMNZ BS496
<i>Fustiaria rubescens</i> (Deshayes, 1825)		Piran
<i>F. sp.</i>		NMNZ sand lagoon
Rhabdidae		
<i>Rhabdus rectius</i> (Carpenter, 1864)		San Juan Islands USNM 678797
Laevidentaliidae		
<i>Laevidentalium callipapulum</i> (Dall, 1889)		USNM 765451
Entaliniidae		
<i>Entalina quinquangularis</i> (Forbes, 1843)		Thalassa Y 378 ¹ Bergen, Norway*
<i>Bathoxiphus ensiculus</i> (Jeffreys, 1877)		Incal WS 01 ¹
<i>B. sp.</i> S 153		NMNZ SI53
<i>Heteroschismoides subterfissum</i> (Jeffreys, 1877)		Nordatlante 85 ¹ ES 218 ²
Pulsellidae		
<i>Pulsellum lofotense</i> (M. Sars, 1865)		Bergen, Norway*
<i>P. salishorum</i> Marshall, 1980		San Juan Islands
<i>P. sp.</i> BS 940		NMNZ BS940
<i>P. sp.</i> P 937 I		NMNZ P937 I
<i>Annulipulsellum euzkadii</i> Scarabino, 1986		BIOGAS IV, DS63 ¹
<i>Striopulsellum sanderi</i> Scarabino, in lit.		Nordatlante P19 ¹
Siphonodentaliidae		
<i>Siphonodentalium grandis</i> (Verill, 1884)		Incal WS 02 ¹
<i>S. lobatum</i> (Sowerby, 1860)		ESI64 ²
<i>S. spectabilis</i> Verill, 1885		Incal DS 09 ¹
<i>S. vitreum</i> M. Sars, 1851		Greenl. Exp. 42 ³
Wemersoniellidae		
<i>Wemersoniella turnerae</i> Scarabino, 1986		Incal WS 07 ¹
Gadilidae		
<i>Gadila fraseri</i> Nickles, 1979		Galathea St. 101 ³
<i>G. metivieri</i> Scarabino, in lit.		BIOGAS V, DS66 ¹
<i>G. sp.</i>		NMNZ P939
<i>Cadulus aberrans</i> Whiteaves, 1887		Monterey Bay
<i>C. arctatus</i> Jeffreys in Locard, 1898		Thalassa Z 407 ¹
<i>C. cylindricus</i> Jeffreys, 1877		ES 27 ²
<i>C. delicatulus</i> Suter, 1913		NMNZ BS544
<i>C. jeffreysi</i> (Monterosato, 1875)		BIOGAS V, DS66 ¹
<i>C. propinquus</i> Sars, 1878		BIOGAS VI, DS82 ¹
<i>C. subfusiformis</i> (M. Sars, 1865)		Bergen, Norway*
<i>C. sp.</i> 68		USNM 803468
<i>C. sp.</i> P927 I		NMNZ P927 I

fixative produced good fixation of cytoplasmatic components in small specimens and pieces of tissue. Bouin's fluid (aq.) penetrates tissues rapidly, decalcifies, but often damaged cilia-tion. This fluid was used also for decalcification after one of the other fixatives. Fixation times ranged from 2-24h. The specimens were washed in 70% ethanol + NH₄ (3 drops/100ml) to remove the picric acid. After dehydration in ethanol, tissues were transferred to methyl benzoate as an antemedium, benzol, 40% paraffin and embedded in 58-60°C Paraplast. Sections of 5-7 μ m were taken and stained with Azocarmine and Anilineblue-Orange G after Heidenhain (AZAN), Haematoxylin and Eosin after Mayer (HE) or Kernechtrot + Picroindigocarmine (KP). Recipes for fixation and staining were modified after Adam and Czihač (1964). Museum samples, usually kept in alcohol, were treated in the same way starting with decalcification or dehydration.

SEM PREPARATION

The same fixatives were used as for histological purposes. Tissues were dehydrated in grades of acetone, critical point dried with liquid CO₂ and sputter coated with a 200 Å gold-layer. The samples were examined in a Jeol JMS 09 scanning electron microscope.

TEM PREPARATION

Two methods were applied as follows: 1) 4 hr in 3% glutaraldehyde in phosphate buffer 0.1M pH 7.2-7.4 (after Sorensen) or cacodylate buffer 0.1M pH 7.2; postfix for 2 hr in 2% OsO₄ 0.1M in the corresponding buffer. To 100ml 0.1M buffer, 10g sucrose were added to adjust the osmolarity for sea-water. 2) 2 hr fixation in glutaraldehyde-paraformaldehyde (1% formalin + 2.5% glutaraldehyde in 0.1M cacodylate buffer pH 7.2) and postfix for 2 hr in 2% OsO₄ in the same buffer. All fixatives were cooled to 4°C. Ethylen diamine tetra acetic acid (EDTA) was used for decalcification. After dehydration in ethanol the samples were embedded either in Epon-Araldite (Mollenhauer, 1964) or in Spurr's medium (Spurr, 1969). Semi-thin sections (0.5-1 μ m) were stained with 0.1% toluidine blue solution. Sections of 70-80 nm were made on a Reichert OMU-3 microtome with glass or diamond knives, and treated for 45 min with a saturated uranyl-acetate solution (Watson, 1958) and for 5 to 8 min with a 0.4% solution of lead-citrate (Venable and Coggeshall, 1965). Sections were studied under a Zeiss EM9/S2 transmission electron microscope.

RESULTS

ANTERIOR MANTLE EDGE

GENERAL CHARACTERISTICS

The anterior pallial opening is supported by a ring of cartilage-like connective tissue as well as by muscles. It can

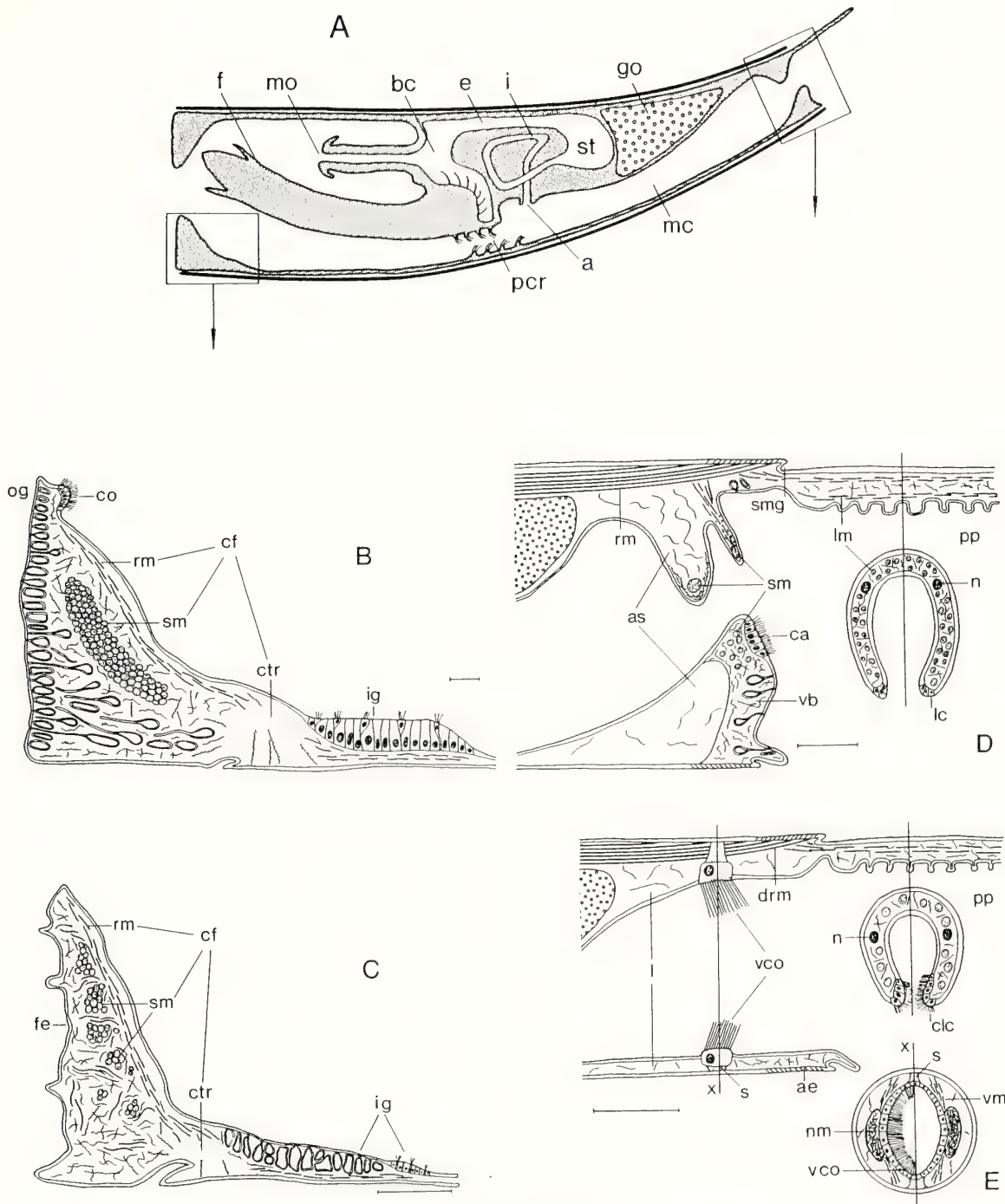


Fig. 1. **A.** Scaphopod in a schematic longitudinal section; **B.** Anterior mantle edge in the order Dentaliida (e.g. *Antalis*), semischematic longitudinal section; **C.** Anterior mantle edge in the order Gadilida (e.g. *Pulsellum*), semischematic longitudinal section; **D.** Posterior mantle edge in the order Dentaliida (e.g. *Antalis*), semischematic longitudinal section, insert (not to scale) showing cross-section of the pavillon proper; **E.** Posterior mantle edge in the order Gadilida (e.g. *Entalina*), semischematic longitudinal section, inserts (not to scale) showing cross-sections of the pavillon proper and the valve ciliary organ (ciliation drawn on left side only); a, anus; ae, attachment epithelium; as, annular sinus; bc, buccal cavity; ca, ciliated area; cf, central fold; clc, ciliated ledge cells; co, ciliary organ; ctr, connective tissue ring with periostracal groove; drm, dorsal retractator muscle; e, esophagus; f, foot; fe, frontal epithelium; go, gonad; i, intestine; ig, inner gland region; l, lacunae; lc, ledge cells; lm, longitudinal muscles; mc, mantle cavity; mo, mouth opening; n, nerve; nm, neuropil; og, outer gland region; pcr, preanal ciliary ridges; pp, pavillon proper; rm, radial muscles; s, socket of connective tissue; sm, sphincter muscle; smg, supramarginal groove; st, stomach; vb, ventral bolster; vco, valve ciliary organ; vm, valve muscle; x, identifies axis of cross-section in lower insert of E; scale bars = 100 μ m.

exhibit different gland regions. The whole complex (see Figs. 1A-C, 2) can be divided into: 1) a frontal epithelium; 2) a central fold; 3) an inner gland region.

In all scaphopods the central fold consists of a ring of connective tissue and the muscle apparatus. The former is a wedge-shaped mass of extracellular matrix in which collagen and scaffolding fibers (fibers grillagées) are embedded (Gabe and Prenant, 1950) (Fig. 3). The orientation of the fibers is predominantly radial. The term "cartilage-like" indicates the histological and functional resemblance of this tissue to vertebrate cartilage; it does not indicate homology.

Towards the anterior aperture, collagen and muscle fibers become more abundant, replacing the extracellular matrix. Thus, the cartilage-like appearance is lost (Figs. 2, 4).

This ring provides an attachment site for the pallial muscles on both proximal and distal sides. The periostracal groove of the outer mantle epithelium has the shape of a concentric ring. Thus, constant shape and diameter are maintained by connective tissue for the shell-forming epithelia. In fixed animals the anterior mantle margin is always retracted, so the groove opens to the anterior (Figs. 1, 4). Towards the anterior aperture the central fold includes a

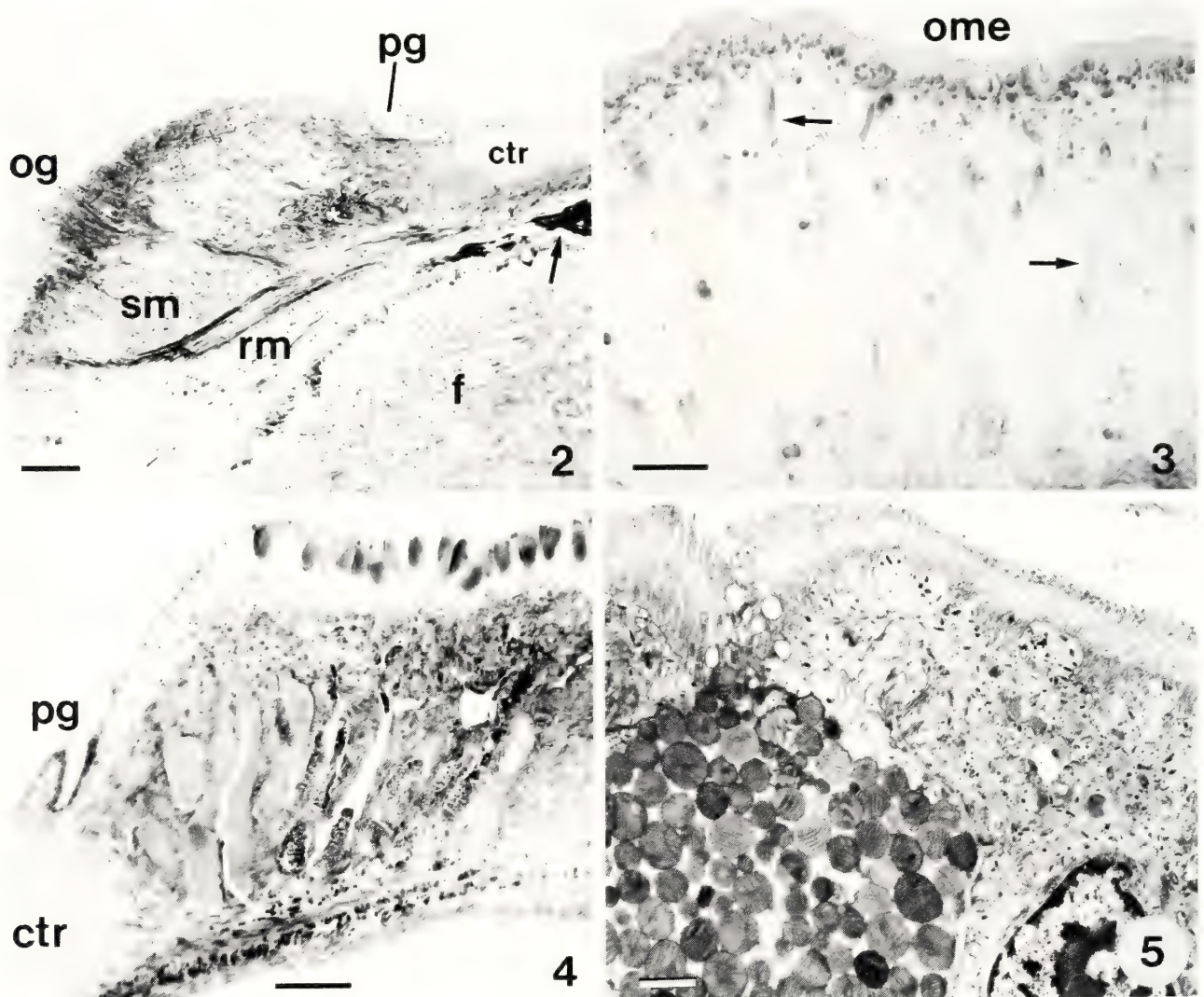


Fig. 2. *Antalis inaequicostatum*, anterior mantle edge in longitudinal section (formalin, AZAN, 7 μ m); note the well developed fibrous connective tissue (asterisk); ctr, connective tissue ring; f, foot; og, outer gland region; pg, periostracal groove; rm, radial muscles; sm, sphincter muscle; inner gland region (arrow); scale bar = 100 μ m. **Fig. 3.** *Fustiaria rubescens*, cross-section of the connective tissue ring in the anterior mantle edge (Bouin, AZAN, 7 μ m); note the homogeneous intercellular matrix, where radially arranged collagen fibres (arrows) are embedded; ome, outer mantle epithelium; scale bar = 10 μ m. **Fig. 4.** *Antalis dentalis*, longitudinal section of the outer gland region showing the different types and sizes of subepithelial gland cells (Bouin, AZAN, 7 μ m); ctr, connective tissue ring; pg, periostracal groove; scale bar = 25 μ m. **Fig. 5.** *Antalis dentalis*, TEM micrograph of the outer gland region in longitudinal section (glutaraldehyde - OsO₄, Epon-Araldite); the subepithelial gland cell contains granules of various density and with substructures; scale bar = 1 μ m.

sphincter muscle that can regulate the width of the opening (Fig. 1B). Radially arranged dilator muscles function as antagonists and longitudinal fibers beneath the inner epithelium pull the fold back into the mantle cavity (Figs. 1B-C, 2). Other muscle fibers from the mantle are attached to the base of the supporting ring to retract the entire organ.

The dorsal region of the organ is innervated by a pair of cerebral nerves, the ventral portion by a pair of pleural nerves. Presence and differentiation of gland cells and sensory receptors differ within and between the orders.

DENTALIIDA

In the order Dentaliida all three components of the anterior mantle margin are developed. A detailed description representative for most dentaliid genera is given for *Antalis*, the genus with three species available for TEM studies (Table 1). *Fustiaria* (= *Pseudantalis*) *rubescens* (Deshayes); for nomenclatural considerations (see Emerson, 1951, 1952) is the only species for comparison on the ultrastructural level. Light microscopical differences between the other genera are listed in Table 2.

FRONTAL EPITHELIUM

The frontal epithelium covers the vertical surface of the anterior mantle margin facing the environment. In the Dentaliida it is identified as a glandular structure by the presence of subepithelial gland cells. It is referred to as the outer gland region. Two types of epithelial cells can be distinguished: 1) "normal" epithelial cells of 6-10 μm height with relatively electron-dense cytoplasm and a basally located nucleus; 2) cells with less dense cytoplasm and a lower density of organelles. These cells are most clearly distinguished from type 1 by foldings of the basal membrane which sometimes reach the upper quarter of the cell body. Numerous small vesicles can be seen on either side of the membrane. All cells are covered with 2-2.3 μm long microvilli and connected to each other by desmosomes and to the basal lamina by hemidesmosomes.

The subepithelial gland cells characterize the appearance of the entire dentaliid anterior mantle margin and occur in two forms (Fig. 4): barrel-shaped gland cells of 70-110 μm length can be distinguished from bottle- or flask-shaped cells that reach as far as 500 μm down into the mesenchyme of the pallial edge. The latter communicate with the surface only by long, thin ducts. Both cell types have basally located nuclei with large nucleoli. In histological sections the mucus stains from red to violet with AZAN and greenish orange with KP. Frequently the cell contents are continuous with the mucus layer covering the frontal pallial epithelium. In TEM sections the cell contents consist of spherical, membrane-bound vesicles of about 1 μm diameter. In *Antalis* spp. they show a sub-structure of straight or curved lines (Fig. 5), which is absent in *Fustiaria rubescens*. Within the same

cell, electron density of the vesicles can vary.

In longitudinal section the anterior mantle margin is somewhat triangular. In the Dentaliida the tip representing the pallial orifice carries the section of an annular ciliary organ (Figs. 1B, 6). A ring of 5-6 (*Fustiaria*) or 6-8 (*Antalis*) rows of prismatic, multiciliary cells (Fig. 7) lines the lumen of the opening, especially if the latter is contracted or closed. The nuclei are found in the proximal half of the cell, and frequently a large nucleolus is present. The cytoplasm is packed with mitochondria. Kinocilia of 15 μm length show the common 9x2+2 pattern and a basal plate. A striated rootlet (banding 56nm) is attached to the basal body at an angle of 25° to the ciliary axes. The membranes of neighboring cells are strongly interdigitated and are connected to each other by desmosomes at or close to the apex.

The ciliary organ was found in all Dentaliida examined except for *Rhabdus rectius* (Carpenter). Here, instead, two dorsolateral slits are formed by the epithelium of the distal outer gland region (Figs. 8, 9, 10A). The floor of these more or less tubular invaginations is covered by cells resembling epithelial cells, whereas the epithelium of the walls contains at least two types of specialized cells (Fig. 10B):

1) Highly columnar cells of 50 μm height with long microvilli, a nucleus close to the basal lamina, and cytoplasm that stains faint-blue with AZAN. These cells are arranged in two rows, one on each lateral wall, close to the bottom of the slit.

2) Columnar, sometimes very slender, multiciliary cells 20-30 μm high forming similar rows right above type 1. Their nuclei also lie basally, but take the red stain better than those of the first type. The cytoplasm is less dense and contains many evenly distributed dark granules. Apically, the basal bodies of the cilia appear as a line parallel to the cell membrane. These can be accompanied by gland cells that could represent a third cell type. Histologically, the outer and inner gland regions are comparable to those in the other members of the order.

RECEPTOR CELLS

The outer gland region and the epithelium of the central fold are marked by the presence of ciliary sensory receptors. In the three species of *Antalis* ultrastructurally examined, two types can be distinguished.

Type 1 (Figs. 11A, 12, 13), very abundant in *Antalis* spp., represents a swollen process of a nerve cell. This sensory cell is more or less cylindrical with a maximum diameter between 2.4 μm and 3.8 μm . No nucleus was found. It is, therefore, thought to lie in the perikaryon of the nerve cells in the underlying mesenchyme. The dendritic processes were traced only for short distances beneath the epidermis. The cytoplasm appears electron lucent except for scattered neurotubules. Close to the epithelial surface several large, elongated mitochondria can be found. They adjoin the

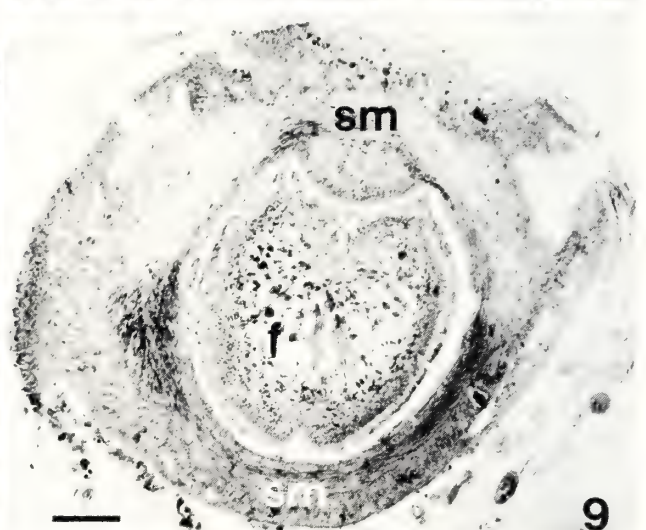
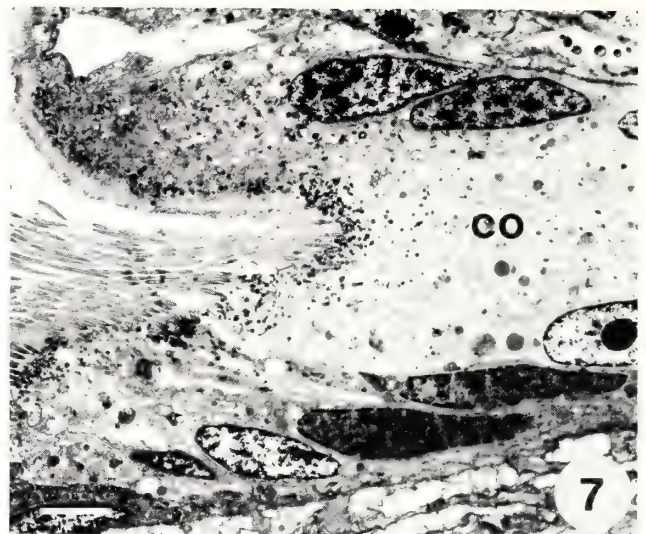
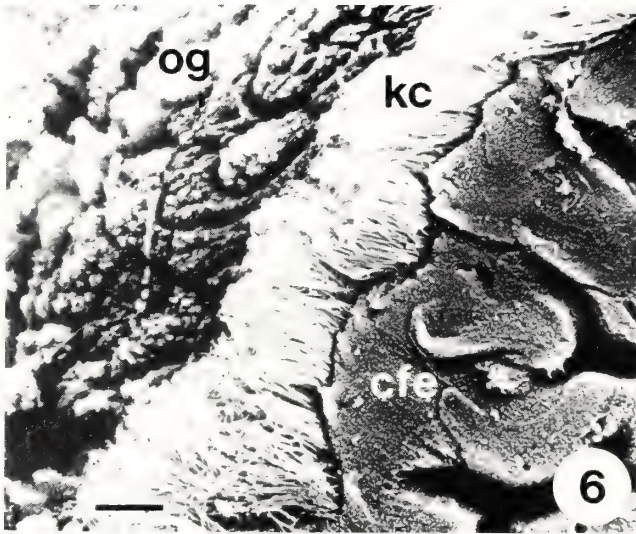


Fig. 6. *Fustiaria rubescens*, SEM micrograph of the anterior mantle edge showing the ciliary organ; cfe, central fold epithelium; kc, kino-cilia; og, outer gland region; scale bar = 5 μ m. **Fig. 7.** *Antalis occidentalis*, TEM micrograph of the ciliary organ in cross-section (glutaraldehyde - OsO₄, Epon-Araldite); note the abundance of dark granules close to the cell apices; co, cells of the ciliary organ; scale bar = 5 μ m. **Fig. 8.** *Rhabdus rectius*, SEM micrograph of the anterior mantle edge in dorso-frontal view; note the slit-like invaginations; pg, periostracal groove; scale bar = 300 μ m. **Fig. 9.** *Rhabdus rectius*, cross-section of the anterior mantle edge with the dorso-lateral slits (formalin, AZAN, 7 μ m); f, foot; sm, sphincter muscle; scale bar = 250 μ m.

centrioles of the sensory cilia. The number of cilia varies from 5 to 25, but the higher number is more common. With a length of 2-4 μ m, the cilia are shorter than those of the ciliated epithelial cells of the central fold. The bases of the receptors are slightly sunken in, although the cell apices can protrude over the other epithelial cells. Some scanning electron microscopographs reveal tapering cilia, suggesting reduction of pairs of microtubules towards the tip. Connections to neighboring cells are effected by desmosomes and septate junctions.

Type 2 represents a collar-receptor (Figs. 11C, 14, 15), which is much less abundant in *Antalis* spp. than in *Fustiaria rubescens*. The dendritic process is more slender than that of type 1, measuring 1-2 μ m across. One or two collar struc-

tures have been observed for one sensory cell. One 5-6 μ m long cilium is surrounded by 8 modified microvilli, so-called stereomicrovilli (Haszprunar, 1985; Salvini-Plawen, 1988), which are basally connected by a membrane. Many filaments can be observed within the stereomicrovilli, especially in the region adjacent to the cilium.

In *Fustiaria rubescens*, type 1 is rare and predominantly found in the outer gland region. Type 2, the collar-receptor, is more common and found mainly in the epithelium of the central fold. Both receptor types are similar in all aspects to the respective types in *Antalis* spp. Another kind of receptor cell, here termed type 1a (Figs. 11B, 16), appears in both epithelial regions. It has a diameter of about 1.5 to 2 μ m and the cytoplasm is packed with ellipsoidal

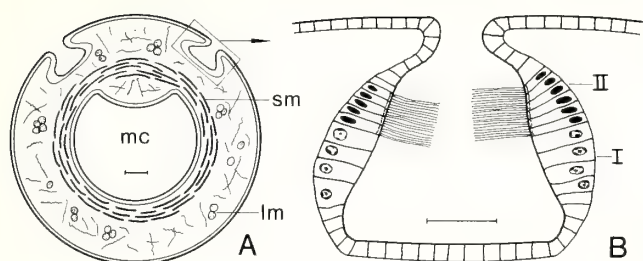


Fig. 10. *Rhabdus rectus*. **A.** semischematic cross-section of the anterior mantle edge; scale bar = 300 μm ; **B.** detail from A. showing the epithelium of the dorso-lateral slit; scale bar = 200 μm .

mitochondria ($0.4 \times 0.6 \mu\text{m}$). Apically, 4-5 cilia of normal structure can be seen. A tiny rudiment of a rootlet inserts at the single centriole.

INNER GLAND REGION

The inner gland region, a differentiation of the inner pallial epithelium, terminates the complex of the anterior mantle edge proximal to the central fold. It forms a tube of epithelial, mucus-secreting cells of variable extent, probably depending on the state of contraction of the underlying mantle muscles. The barrel-shaped epithelial gland cells (Fig. 17), measuring approximately $40\text{-}90 \times 20 \times 20 \mu\text{m}$, are filled with densely packed vesicles of light- and electron lucent appearance, which have the tendency to coalesce to a single droplet of mucus. The nucleus is generally located close to the thin basal lamina. Another type of gland cells, restricted to few specimens of *Antalis* species only, is flask-like with

a larger basal portion and a thin process passing between the other cells to reach the surface (Fig. 2). Their contents vary from violet to bright red with AZAN and greenish orange in KP. At present, it is not clear whether these cells are epithelial or subepithelial. Apart from these, no subepithelial gland cells were recognized in the inner gland region of Dentaliida. The surface of the gland cells is partly covered by ciliated epithelial cells with apically located nuclei (Fig. 17). These cells are attached to the basal lamina by very slender processes only.

GADILIDA

The anterior pallial edge in Gadilida has the same basic structure as in dentaliids, but the entire organ has undergone different elaboration (Fig. 1C). The ring of cartilage-like connective tissue is generally not as extensive as in the Dentaliida. In some species only a narrow band at the base of the periostacal groove is differentiated. Plate (1892) and Odhner (1931) noted the absence of the outer gland region in members of this order. The ciliary organ of the central fold is absent.

FRONTAL EPITHELIUM

Characteristic for the gadilid frontal epithelium is the elaboration of papillae. Their number is high in the representatives examined of the families Entalinidae, Pulsellidae and Wemersoniellidae. In Siphonodentaliidae and Gadilidae, however, papillae are more scattered. In *Entalina quinquangularis* (Forbes), papillae are 15 and 20 μm high. Depending on the state of contraction the conical protrusions

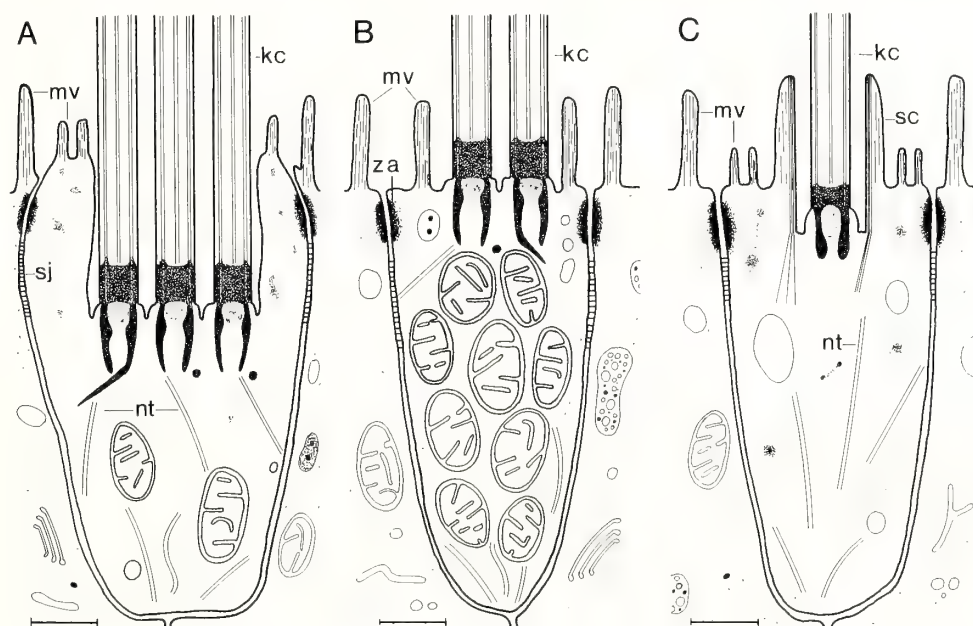


Fig. 11. Reconstruction of ciliary sensory cells of the anterior mantle edges. **A.** Type 1 of *Antalis*; **B.** Type 1a of *Fustiaria*; **C.** Type 2 (collar receptor) of *Antalis*; kc, kinocilium; mv, microvilli; nt, neurotubules; sj, septate junction; za, zonula adherens; scale bars = 0.5 μm .

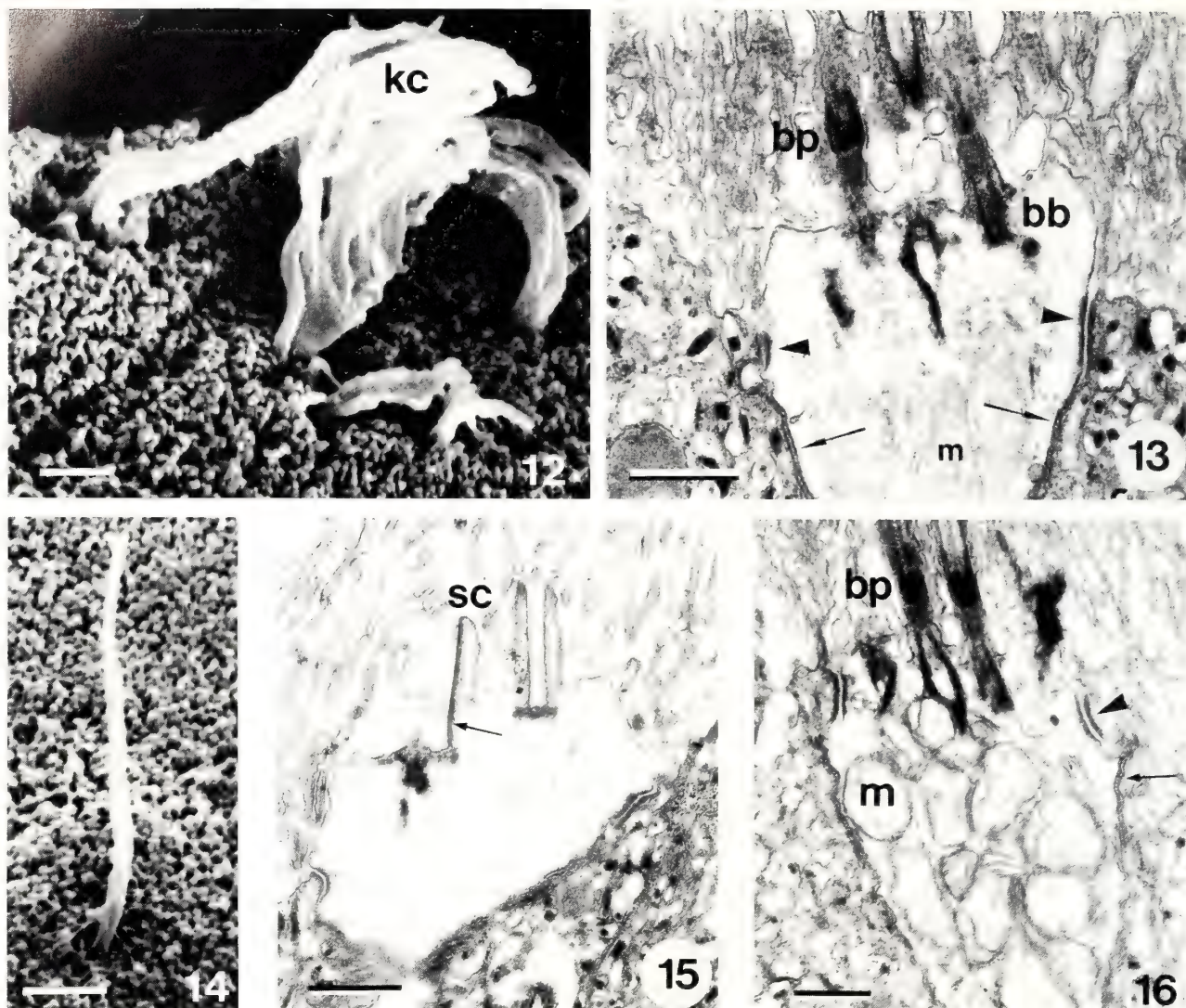


Fig. 12. *Antalis occidentalis*, SEM micrograph of a ciliary sensory cell of type 1 in the frontal epithelium of the anterior mantle edge; kc, kinocilia; scale bar = 0.5 μ m. **Fig. 13.** *Antalis occidentalis*, TEM micrograph of a type 1 receptor in a longitudinal section (glutar-paraformaldehyde - OsO₄, Spurr); bb, basal body; bp, basal plate; m, mitochondrion; septate junctions (arrows), zonulae adherentes (arrow-heads); scale bar = 0.5 μ m. **Fig. 14.** *Antalis occidentalis*, SEM micrograph of a type 2 receptor (collar receptor) of the frontal epithelium; scale bar = 0.5 μ m. **Fig. 15.** *Antalis dentalis*, TEM micrograph of a type 2 receptor in a longitudinal section (glutaraldehyde - OsO₄, Epon-Araldite); note the two collar structures; sc, stereocilia (=stereomicrovilli); dense microfilaments in a stereocilium (arrow-head); scale bar = 0.5 μ m. **Fig. 16.** *Fustiaria rubescens*, TEM micrograph of a type 1a receptor cell in the frontal epithelium (glutaraldehyde - OsO₄, Epon-Araldite); bp, basal plate; m, mitochondrion; septate junction (arrows); zonula adherens (arrow-head); scale bar = 0.5 μ m.

are either approximately 30 μ m apart, so they are elaborated on a flat base of tissue close to each other (Figs. 18, 19). Generally, papilla density is highest close to the mantle opening and decreases towards the periphery.

The transition from the frontal epithelium to the outer mantle surface is marked by a crest of connective tissue (Figs. 18, 19). The periostracal groove is visible some distance below the crest on the outside of the mantle. In fixed specimens the frontal epithelium is sunken in, giving the mantle margin the appearance of a crater rimmed by the above-mentioned crest.

The frontal epithelium cells are rather flat - 3 μ m in *Cadulus subfusiformis* (M. Sars), 5 μ m in both *Entalina quinquangularis* (Forbes) and *Pulsellum lofotense* (M. Sars). The latter also possess vesicle containing cells which appear to be epithelial gland cells.

An unusual tissue is located beneath the frontal epithelium of *Entalina quinquangularis*. Being only about 15 μ m thick, this layer is poorly visible in histological sections (Fig. 19, x). The tissue consists of spherical, vacuolized cells that proved to be problematic in TEM preparations, because their contents, perhaps of mucoid nature, were washed out.

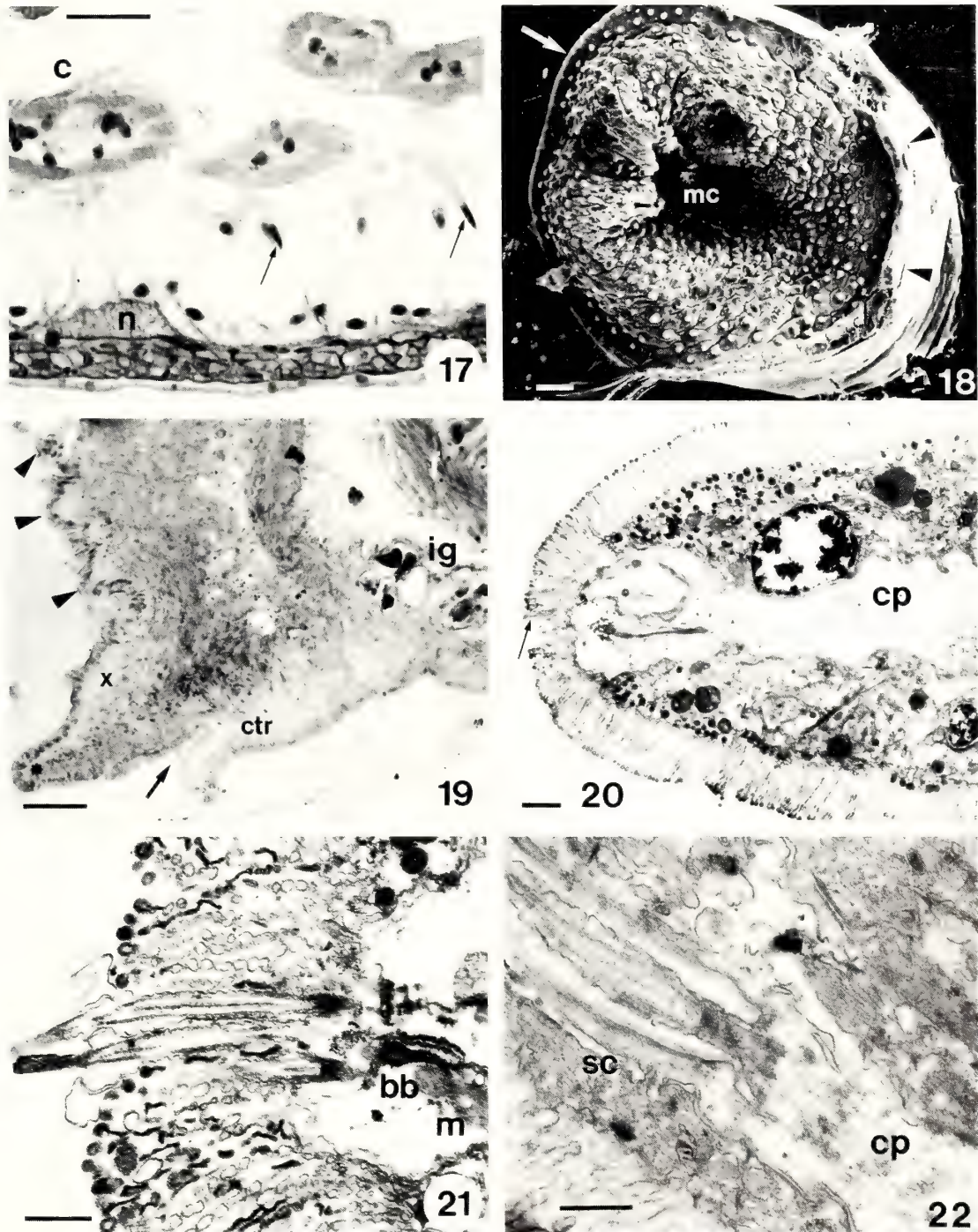


Fig. 17. *Fustiaria rubescens*, cross-section of the inner gland region (Bouin, AZAN, 7 μ m) showing epithelial gland cells; note the position of the nuclei of the ciliated epithelial cells (arrows); c, captaculum; n, pallial nerve; scale bar = 25 μ m. **Fig. 18.** *Entalina quinquangularis*, frontal view of the anterior mantle edge in a SEM micrograph (glutaraldehyde - OsO₄, Spurr); note the numerous papillae; mc, opening of the mantle cavity; periostacal groove (arrow-heads); rim of the "crater" (arrow); scale bar = 100 μ m. **Fig. 19.** *Entalina quinquangularis*, anterior mantle edge in longitudinal section (Bouin, AZAN, 7 μ m); note the less voluminous ring of turgid connective tissue (ctr); f, foot; ig, inner gland region; x, mucoid tissue layer; papillae (arrow-heads); periostacal groove (arrow); rim of the "crater" (asterisk); scale bar = 100 μ m. **Fig. 20.** *Entalina quinquangularis*, TEM micrograph of a frontal epithelial papilla in longitudinal section (glutaraldehyde - OsO₄, Spurr); cp, sensory cell process; cilium of the apical sensory cell (arrow); scale bar = 1 μ m. **Fig. 21.** *Entalina quinquangularis*, ciliary sensory cell at the tip of a papilla (glutaraldehyde - OsO₄, Spurr); bb, basal body; m, mitochondria; scale bar = 0.5 μ m. **Fig. 22.** *Cadulus subfusiformis*, pitted ciliary sensory cell (type 2, collar-receptor) at the tip of a papilla (glutarparaformaldehyde - OsO₄, Spurr); cp, sensory cell process with neurotubuli; sc, stereocilium (=stereomicrovillus); scale bar = 0.5 μ m.

Delicate fibers of connective and muscle tissue fill the intercellular spaces. For other members of the family Entalinidae, available from museum samples only, this tissue could not conclusively be found.

RECEPTOR CELLS

Although some type 1 receptor cells were found in the frontal epithelium of *Cadulus subfusiformis*, the sensory elements of the gadilid anterior mantle margin are found in the papillae. Each papilla carries one or more sensory processes of neurons (Fig. 20), whose perikarya lie in the connective tissue beneath. A connection between a receptive process and such a perikaryon was not found. Nor was it possible to trace the process of the sensory cell beneath the base of the papilla. The cytoplasm appears optically empty and only neurotubules can be seen orientated mainly in the direction of the cell axis. Two or more cilia (9x2+2 pattern) protrude from the apex (Fig. 21). A basal plate and a single centriole form the anchoring apparatus. No rootlet is developed, but small bundles of fibers spread from the centriole to the surrounding mitochondria (Fig. 21). The mitochondria are elongated and measure about 1x0.4 μ m. Some similarity to the type 1 receptors of dentaliids is evident. Papillae with collar-receptors appear in *Cadulus subfusiformis* (Fig. 22).

INNER GLAND REGION

The inner gland region in Gadilida is mainly formed by subepithelial gland cells. Accordingly, the cell bodies, apart

from the secretory duct, are located beneath the basal lamina. Like other areas of the mantle cavity the inner gland region is covered by a cuboidal epithelium. The form of the gland cells, influenced by the packing between the basal lamina and the pallial muscles, is cuboidal to honeycomb-like. Frequently, they over- and underlay each other (Figs. 23, 24).

Staining with AZAN reveals two types of subepithelial secretory cells: one staining violet to red, the other taking no stain (Fig. 23). In TEM-sections both types contain small vesicles that show tendencies to coalesce to larger secretion vacuoles. A cell can be completely filled with a single, membrane-bound droplet (Fig. 24). Electron density seems to parallel the histological staining properties. The nucleus with a large nucleolus usually lies near the base of the cell. The cytoplasm surrounding the nucleus is rich in golgi complexes.

The proximal border of the gland region is formed by a few rows of epithelial gland cells. They are prismatic in shape and stain in a faint blue or remain unstained with AZAN. Unfortunately, this tissue is easily damaged by improper fixation, processing, or long storage; therefore, it is usually not preserved in museum material. Table 2 lists the characters of the anterior mantle margin for the examined genera.

EPITHELIUM OF THE MANTLE CAVITY

Most of the pallial cavity is lined by flat epithelial cells.

Table 2. Characters of the anterior mantle margin: (++) abundant; (+) present; ($\pm$) scarce, few; (-) absent; (?) no information.

	Frontal Epithelium				Ciliary Organ		Receptor Cell Types				Inner Gland Region		
	subepith. gland cells	epith. gland cells	papillae	"mucoid" tissue	cell rows	slits	1	la	2	papil- lae	subepith. gland cells	mucoid epith. gland cells	dark epith. gland cells
<i>Antalis</i>	++	-	-	-	6-8	-	+	-	$\pm$	-	-	+	+
<i>Dentalium</i>	++	-	-	-	6-8	-	?	?	?	-	-	+	-
<i>Fissidentalium</i>	++	-	-	-	6-8	-	?	?	?	-	-	+	-
<i>Graptacme</i>	++	-	-	-	?	-	?	?	?	-	-	+	-
<i>Fustiaria</i>	++	-	-	-	5-6	-	$\pm$	+	+	-	-	+	-
<i>Laevidentalium</i>	++	-	-	-	+	-	?	?	?	-	-	+	-
<i>Rhabdus</i>	++	-	-	-	-	+	?	?	?	-	-	+	-
<i>Entalina</i>	-	$\pm$	++	+	-	-	-	-	-	+	+	+	-
<i>Bathoxiphus</i>	-	?	++	?	-	-	?	?	?	?	+	+	-
<i>Heteroschismoides</i>	-	?	++	?	-	-	?	?	?	?	+	+	-
<i>Pulsellum</i>	-	$\pm$	++	-	-	-	-	-	-	+	+	+	-
<i>Annulipulsellum</i>	-	?	++	-	-	-	?	?	?	?	+	+	-
<i>Striopulsellum</i>	-	?	++	-	-	-	?	?	?	?	+	+	-
<i>Siphonodentalium</i>	-	?	$\pm$	-	-	-	?	?	?	?	+	+	-
<i>Wemersoniella</i>	-	?	++	-	-	-	?	?	?	?	+	+	-
<i>Gadila</i>	-	?	$\pm$	-	-	-	?	?	?	?	+	+	-
<i>Cadulus</i>	-	-	$\pm$	-	-	-	+	-	-	+	+	+	-

The mantle area anterior to the head is characterized by the muscle fibers embedded between the outer and the inner mantle layer. The fibers emerge from the dorsal body retractors. Posterior to the head the musculature decreases conspicuously. Just in front of the cerebral ganglia the two layers separate: the outer layer continues into the dorsal body epithelium, the inner one meets the epidermis of the head. The line of mantle attachment descends posteriorly to a level between the equator and the upper third of the more or less cylindrical body. Behind the muscular part of the mantle the tubules of the digestive gland and, especially in Gadilida, the gonads are spread laterally between the mantle layers.

Lacaze-Duthiers (1856-7) first described numerous transverse ciliated ridges along the anal region. In fact, these ridges are epithelial rings running around the entire pallial

cavity including the body wall. Each ring consists of one row of cells (Figs. 25, 26). In dentaliids the number of rings ranges from 5 in some *Antalis dentalis* specimens to 22 in *Rhabdus rectius*. The average number is between 12 and 15. In the order Gadilida usually four to eight ciliary rings are present. In *Gadila metivieri* Scarabino, however, only one ring could be distinguished, whereas a specimen of *Siphonodentalium vitreum* M. Sars had 15 rings. The number of ridges is probably body-size dependent. The distance between two rows ranges between 10 μm and 40 μm , depending on the state of contraction of the mantle tissue. In some specimens of various species mucus secreting cells could be identified between the ridges. In *Cadulus aberrans* Whiteaves, three to four spherical gland cells with mucoid contents occur in each interval (Fig. 26).

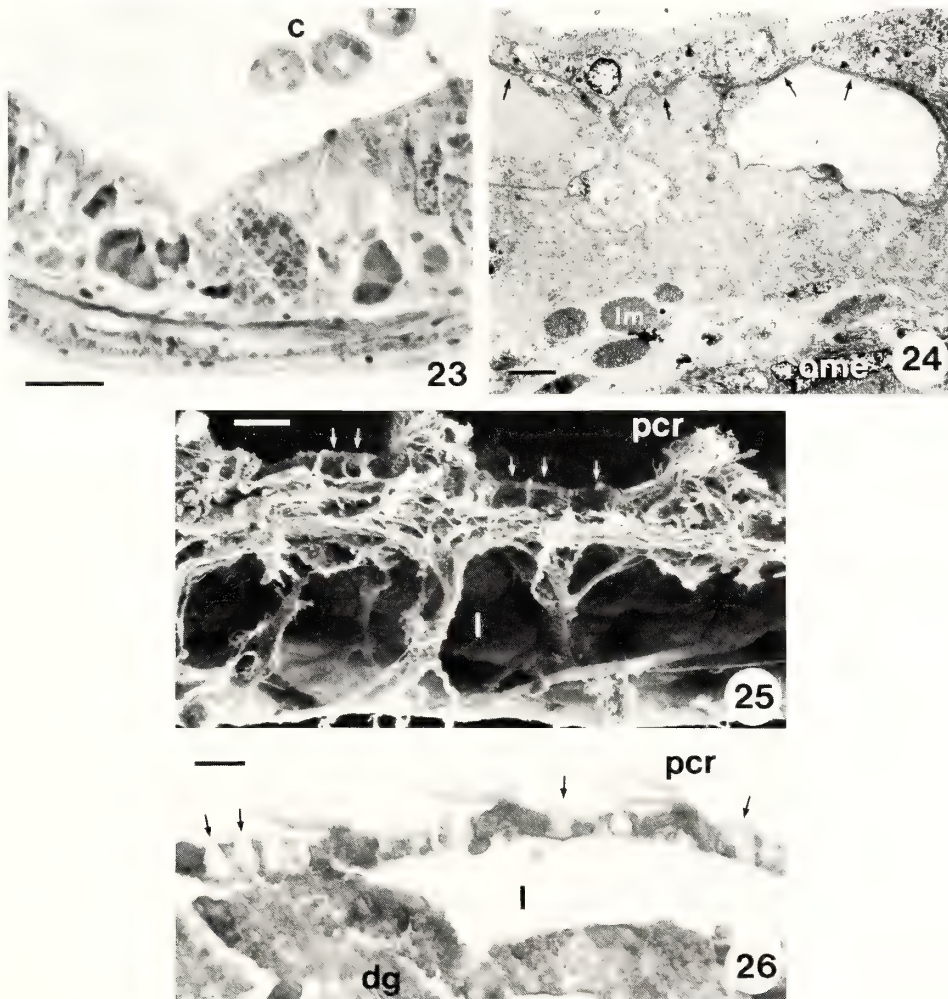


Fig. 23. *Entalina quinquangularis*, longitudinal section (Bouin, AZAN, 7 μm) of the inner gland region, showing subepithelial gland cells; c, captaculum; scale bar = 25 μm . **Fig. 24.** *Entalina quinquangularis*, TEM micrograph of the inner gland region in cross-section (glutaraldehyde - OsO_4 , Epon-Araldite); note the subepithelial gland cells in various stages beneath the basal lamina (arrows); lm, longitudinal pallial muscles; ome, outer mantle epithelium; scale bar = 3 μm . **Fig. 25.** *Antalis dentalis*, SEM micrograph of a longitudinal section of the mantle in the preanal region; l, lacunae; pcr, preanal ciliary ridge; note the epithelial gland cells between the ridges (arrows); scale bar = 10 μm . **Fig. 26.** *Cadulus subfusiformis*, longitudinal section of the same region (glutaraldehyde, AZAN, 7 μm); dg, digestive gland tubule; l, lacunae; pcr, preanal ciliary ridge; epithelial gland cells between the ridges (arrows); scale bar = 5 μm .

In both orders the cilia-bearing cells (30-40 μm wide and 15-20 μm high) protrude conspicuously from the cuboidal mantle epithelium. The densely granulated cytoplasm stains from steel-blue to dark red with AZAN, red with HE and light brown with KP. The nucleus usually lies eccentrically, close to the posterior cell wall. The centrioles form conspicuous lines of strongly refractive granules below the apical cell membrane. Cilia length is approximately twice the height of the cell. In most of the sections the cilia point posteriorly. No ultrastructural work had been done on this mantle region.

Most of the Dentaliida and some Gadilida have a glandular area anterior to the ciliated ridges. Distaso (1906) homologized this area with the hypobranchial gland of other molluscs. The cells resemble those of the inner gland region, albeit much higher (80 μm). These delicate and vulnerable cells easily disintegrate when treated improperly. Thus, no clear evidence is drawn from the analysis of this character.

Spacious blood lacunae are located between the mantle layers below the ciliar ridges (Figs. 25, 26). Towards the posterior mantle region they unite to a single sinus along the ventral midline. The epithelium of the pallial cavity above this blood sinus bulges out.

POSTERIOR MANTLE EDGE

The posterior mantle edge, or pavillon (according to Deshayes, 1825 and Lacaze-Duthiers, 1856-7), is situated at the apical aperture of the shell. Inhalent and, partly at least, exhalent currents pass through this opening. In both scaphopod orders the dorsal retractor muscles have a single ring-shaped attachment site (Fig. 27) on the inner shell surface a short distance from the apex. The pallial orifice represents a valve apparatus to regulate the water flow. The structure and function of this valve-organ differ within Dentaliida and between Dentaliida and Gadilida. Distal to this organ, a dorsal process of the pallial epithelium, the pavillon proper, is differentiated. Different types of gland cells can be distinguished.

The following descriptions are based mainly on histological observations. The situation in the examined species of *Antalis* is representative for all other dentaliid species studied except *Fustiaria* spp. The posterior mantle margin of *Entalina quinquangularis* is described for the Gadilida. The other species of this order do not differ substantially. An interpretation of the functional morphology is provided in the discussion.

ANTALIS

Although Leon (1895) and Boissevain (1904) published descriptions of the histological structure of the pavillon of *Antalis vulgaris* (DaCosta), this organ will be redescribed here. Close to the tip of the shell, where the retractor muscles attach, a ring-like protrusion of the mantle tissue terminates

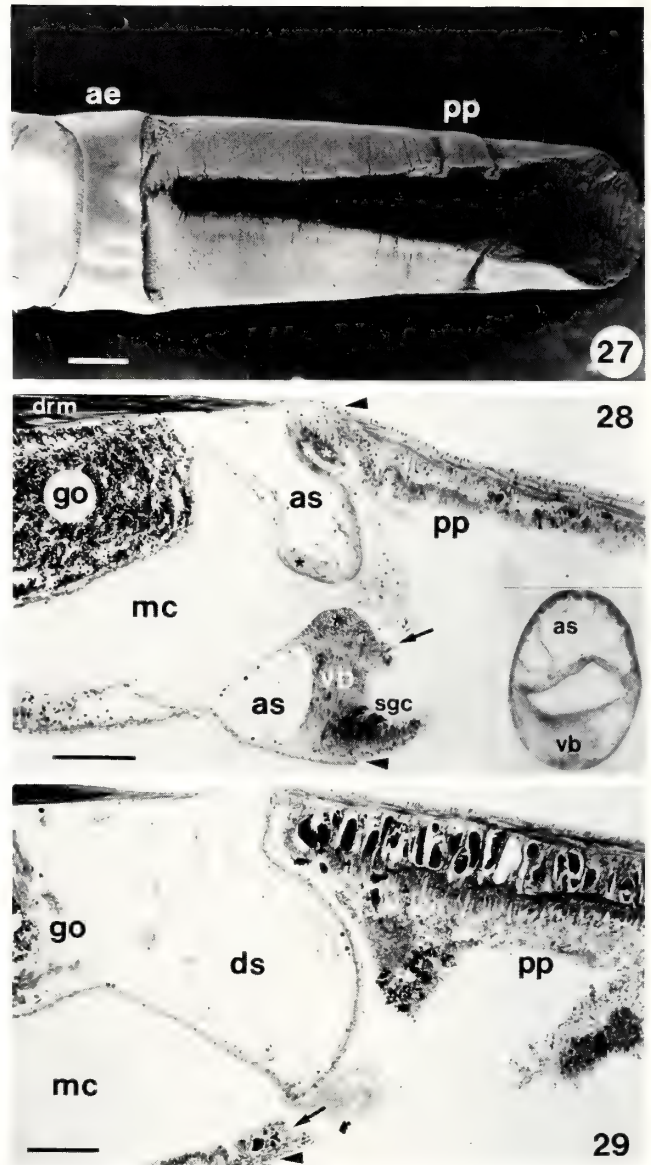


Fig. 27. *Fissidentalium candidum*, SEM micrograph of the posterior mantle edge and the pavillon proper in ventral view; ae, attachment epithelium; pp, pavillon proper; scale bar = 1 mm. **Fig. 28.** *Antalis inaequicostatum*, posterior mantle edge in longitudinal section (Bouin, AZAN, 7 μm); insert (not to scale) shows cross-section; as, annular sinus; drm, dorsal retractor muscle; go, gonad (testis); mc, mantle cavity; pp, pavillon proper; sgc, subepithelial gland cells; vb, ventral bolster; ciliated area (arrow); supramarginal groove (arrow-heads); sphincter muscle (asterisk); scale bar = 100 μm . **Fig. 29.** *Fustiaria rubescens*, posterior mantle edge in longitudinal section (Bouin, AZAN, 7 μm); note the high abundance of subepithelial gland cells in the pavillon proper; ds, dorsal sinus; go, gonad; mc, mantle cavity; pp, pavillon proper; ciliated area (arrow); supramarginal groove (arrow-heads); scale bar = 100 μm .

the pallial cavity. The base of this annular fold is formed by a huge annular blood sinus, which connects the haemolymph spaces of the gonad region with the mid-ventral lacuna of the mantle (Figs. 1D, 28). The dorsal part of the fold is produced

entirely by this sinus. On the ventral side, however, the sinus is underlain posteriorly by a crest of connective tissue, the ventral bolster. In histological sections this tissue resembles the fibrous connective tissue of the anterior mantle edge, showing scaffolding and high densities of collagen fibers (Fig. 28). These fibers make the tissue look denser than the anterior counterpart. Although this tissue is voluminous at the bottom of the annular fold, it rapidly decreases laterally and towards the dorsal side. In the blood sinus it is present only as a narrow layer beneath the epithelium. Another branch of this tissue is part of a smaller, distal projection of the pallial wall, previously undescribed (Fig. 28). Several muscle strands extend through the dorsal section of the sinus (Figs. 1D, 28). They originate from the dorsal retractors and insert on the layer of connective tissue. Therefore, the main direction of the fibers is from anterior-dorsal to posterior-ventral. When contracting, these muscles withdraw the upper part of the annular fold and increase the diameter of the opening. The antagonistic force is provided by a bipartite sphincter muscle. It has two dorsal portions that unite ventrally to a single mass. The anterior dorsal portion within the blood sinus appears as a compact, naked bundle closely joined to the supporting tissue of the epithelium (Figs. 1D, 28). The posterior dorsal portion, together with a layer of connective tissue, forms a narrow but rigid semi-annular projection. On the ventral side, the fibers radiate into the ventral bolster. In the ventral section of the sinus only a few, more ligament-like fibers are present.

Subepithelial gland cells are located in the ventral bolsters. They open towards the pavillon on the posteriorly facing wall of the valve apparatus (Fig. 28). These unicellular glands have a red staining, granulated cytoplasm that resembles the dark staining cells of the outer gland region in the anterior mantle edge (Leon, 1895; Boissevain, 1904). They extend about 25 μm deep into the connective tissue. In some sections the second, weakly staining cell type can also be found, although this type is much less abundant than the other. Sometimes, slightly different cells are found in the dorsal region. Their secretory granules are larger and stain bright red. This type is also present in the pavillon proper. An area of ciliated cells is located either on the outer base or on the outer flank of the valve (Boissevain, 1904; Reynolds, 1988). Reynolds (1988) described two type of ciliary receptors in this region. The rest of the epithelium of the ring-fold consists of low-prismatic cells with large nuclei.

The attachment epithelium of the retractor muscles is followed posteriorly by a shallow supramarginal groove (Stasek and McWilliams, 1973), which separates the valve mechanism from the pavillon proper (Figs. 28, 29). This is a dorsally elongated pallial appendage resembling the tip of a syringe needle (Fig. 27). Thus, the opening has an oblique plane facing the ventral side. In cross section the pavillon proper is crescent-shaped. The inner epithelium of the

pavillon is extensively folded, probably a consequence of the contracted longitudinal muscles of this organ. In the cytoplasm, numerous small granules stain a brownish-green color with AZAN. The large nucleus stains red. Subepithelial gland cells with red granules, belonging to one of the two mentioned types, occur in longer but regular intervals. The ledges of the crescent are covered by cells of a different type. They are more columnar, showing dark-red cytoplasm and large, elongated nuclei. Their function is unclear. Scanning electron micrographs (unpublished) showing tufts of cilia indicate a sensory function. The outside of the pavillon is covered by an epithelium of cuboid cells, which is usually not folded like the inner one. No glands or gland openings could be recognized there.

A loose matrix of connective tissue can be observed between the epidermal layers of the pavillon. Muscles, nerves and the gland cells of the inner surface are embedded here. Conspicuous longitudinal muscle fibers insert at the dorsal side of the attachment epithelium. These shorten the pavillon when contracted. A web of more delicate transverse and dorsoventral muscles are responsible for extension. The entire structure is highly motile, although its movements are usually slow. Haemolymph pressure is unlikely to play an important role in pavillon extension because only a minor mid-dorsal lacuna enters from the annular sinus. After a short distance, this blood space branches into numerous small lacunae.

Innervation of this mantle appendage is achieved by a pair of large nerves. Their origin lies in a pair of small ganglia at the base of the pavillon, which receive connectives from the visceral ganglia. The nerves usually extend and wind through the pavillon. Towards the tip of the pavillon they taper in diameter, but are always clearly detectable.

FUSTIARIA

Both species of *Fustiaria* examined show important differences from *Antalis* spp. and other Dentaliida examined in the structure of the posterior mantle edge, particularly with respect to the valve mechanism and the glandular components of the pavillon order (Figs. 29, 30).

The valve is a simple elaboration of a large dorsal blood sinus. It bulges ventrally and thus forms a lid for the mantle pore. No annular blood sinus, sphincter muscle, or ventral bolster of connective tissue are present in specimens of this genus. The large blood space extends from the tip of the gonad to the muscle attachment site. Similar to *Antalis* spp., it is transversed by many small muscle fibers. Their origin is the dorsal body wall and they insert on the thick basal lamina of the ventral and lateral epithelium or the layer of connective tissue beneath. Ventrally, the mantle cavity ends with a slightly thickened rim just behind the attachment epithelium. The rim contains some circular and dorsoventral muscle fibers and connective tissue, which is not comparable

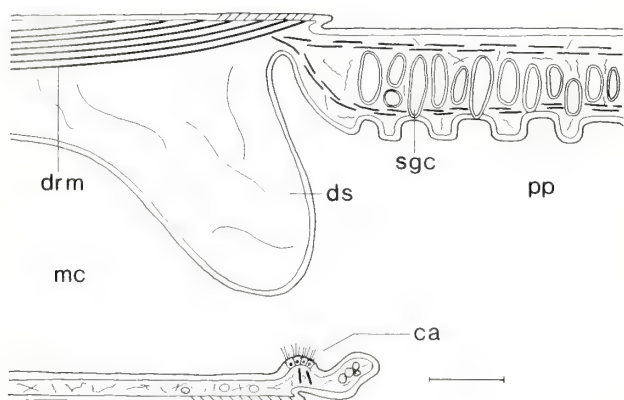


Fig. 30. *Fustiaria rubescens*, semischematic longitudinal section of the posterior mantle edge; note the lacking of the ventral bolster and the high abundance of subepithelial gland cells in the pavillon proper; ca, ciliated area; ds, dorsal sinus; drm, dorsal retractor muscles; mc, mantle cavity; pp, pavillon proper; sgc, subepithelial gland cells; scale bar = 100 μ m.

to the differentiations in *Antalis* spp. Longitudinal sections reveal some ciliated cells belonging to a weakly defined ciliated area of the ventral mantle epithelium. This is probably the equivalent of the more clearly defined area of *Antalis* spp.

With the lack of the ventral bolster, the incorporated gland cells are likewise missing, but the rich development of secretory elements in the inner epithelium of the pavillon proper (Figs. 29, 30) compensates for this. Large gland cells fill the space between the longitudinal muscles of the pavillon throughout the entire length. With AZAN the granules stain from bright red to dark blue and purple (Fig. 29). It is difficult to determine whether they represent different cell types or subsequent physiological stages.

ENTALINA

The apical pallial orifice in the Gadilida can be closed to a vertical slit by a pair of septa arising laterally in the pallial cavity. The slit is closed by the contraction of dorsoventrally orientated muscles lying beneath and on either side of the basal lamina of the epithelium. These muscles, joining in the dorsal and ventral mid-line, insert upon common sockets of connective tissue. Nerves from the small pavillon ganglia descend along the muscle bundles (Figs. 1E, 31). Radial muscle bundles connect inner and outer mantle epithelia.

The epithelium of the slit differentiates a conspicuous ciliary organ (Figs. 1E, 32). It consists of 25 (± 2) large, densely ciliated cells arranged in a single row encircling the lumen of the mantle opening. These cells are about 12 μ m high and 25-30 μ m wide. The large spherical nuclei, often with obvious nucleoli, are located centrally in the cytoplasm. The cytoplasm is packed with mitochondria. Numerous vesicles of different diameters and electron densities are present. Small electron-dense granules can be seen close to the cell apex. The cell surface is covered with tightly packed cilia;

32 centrioles were counted on a single section. The cilia are approximately as long as the cell is high. The basal bodies are strictly aligned, but their structure has not been studied in detail. A long rootlet departs at an angle of about 55° that descends almost down to the basal lamina. The striation from one center of a dark band to the next has an interval of 50-75 nm. The cells rest on a basal lamina of variable thickness. Towards the dorsal and ventral tips of the slit the basal lamina becomes thicker and forms, together with connective tissue fibers, a kind of supporting structure. Beyond the tips both layers, one from each side of the slit, unite to form short peduncles that attach on the outer body wall. In cross section this structure looks like the eye of a needle (Figs. 1E lower insert, 31). Sometimes there is direct contact between a ciliated cell and nervous tissue.

Sensory receptors occur proximal to the ciliary organ. They are probably arranged in two rows, separated by epithelial cells, but this remains to be confirmed. In many aspects these receptor cells resemble those of the anterior mantle edge of the Dentaliida. The nerve process is inflated in the epithelial region and the cytoplasm appears transparent. Five to eight cilia extend their short rootlets to apically located mitochondria. Many filaments and/or tubules can be found in this area. Towards the mesenchyme the cell tapers to a small process, but a direct connection to a perikaryon of a neuron could not be observed.

Distal to the ciliary organ the narrow slit widens to the base of the pavillon proper. The mantle appendage is, as in dentaliids, crescent-shaped in cross section (Fig. 33). Living animals, however, usually show the pavillon as a flat sheet of tissue, which can even be bent outwards. In the inner epithelium the flat cells are usually folded as well, but the large subepithelial gland cells are missing. Instead, secretory cells are present in the epithelium. Their cytoplasm contains various dark granules and vesicles. The apically located nuclei appear very condensed. Such dark and condensed nuclei are found frequently near the epithelium but completely detached from it. This indicates a holocrine secretion modus. Like in the dentaliid genera the pavillon crests are differentiated with regard to their epithelium. A continuous row of three to six ciliated cells run along the ledges (Figs. 33, 34). Cell shape ranges from cuboid to high-prismatic, with a height of 7-12 μ m. The cilia of these cells are about 8 μ m long and of normal structure. The basal apparatus consists of a centriole and, attached to it, a single striated rootlet at an angle of 20° to the ciliary axes. Between the cilia are microvilli of about 2 μ m length. The cytoplasm stains faintly red with AZAN and HE. In TEM sections it appears relatively light and contains mitochondria, rough endoplasmic reticula (RER), golgi vesicles and other vesicles of various sizes and densities. The nucleus is spherical or elongated and situated close to the basal lamina. Frequently, nucleoli are present. At the tip of the pavillon sensory receptors can be recognized between the ciliated cells.

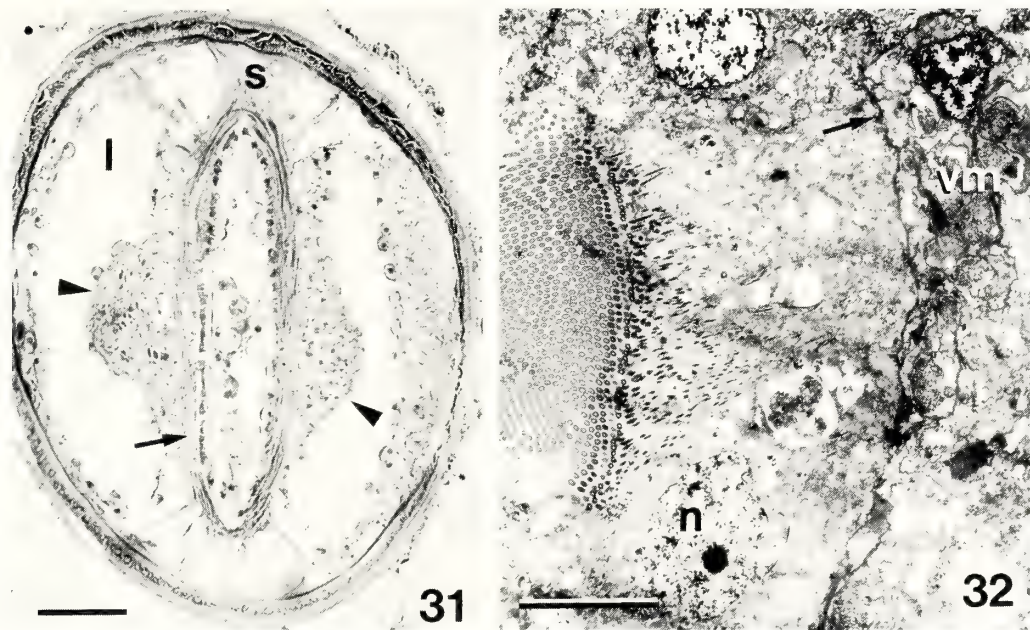


Fig. 31. *Entalina quinquangularis*, cross-section of the posterior mantle edge (formalin, H.E., 7 μ m); l, lacunae; s, ventral socket of connective tissue; fibres of the valve muscle (arrows); crests of nerve- and glia cells (arrow-heads); scale bar = 50 μ m. **Fig. 32.** *Entalina quinquangularis*, TEM micrograph of the valve ciliary organ of the posterior mantle edge, horizontal section (glutar-paraformaldehyde - OsO₄, Spurr); note the long ciliary rootlets descending between the densely packed mitochondria in the ciliated cell; n, nucleus of ciliated cell; vm, valve muscles; basal lamina (arrow); scale bar = 5 μ m.

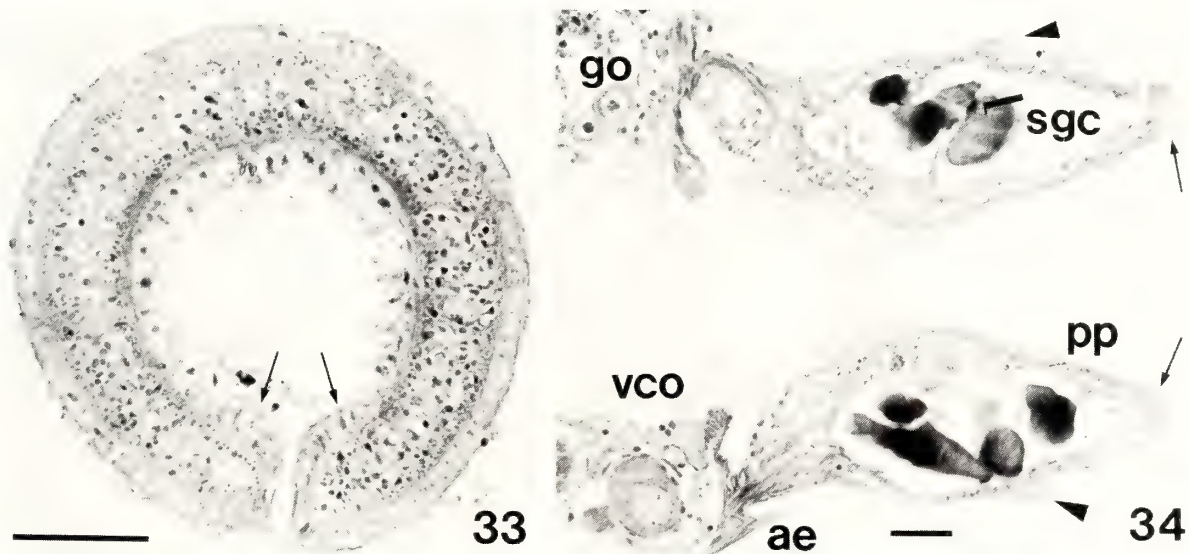


Fig. 33. *Entalina quinquangularis*, cross-section of the pavillon proper (Bouin, AZAN, 7 μ m); ciliated ledge cells (arrows); scale bar = 50 μ m. **Fig. 34.** *Cadulus subfusiformis*, longitudinal section of the posterior mantle edge (Bouin, AZAN, 5 μ m); note the additional ring fold (arrow-heads) and the ciliated ledge cells (arrows); ae, attachment epithelium; go, gonad (ovary); pp, pavillon proper; sgc, subepithelial gland cells; vco, valve ciliary organ; scale bar = 25 μ m.

They are inflated cell processes with the perikaryon below the epidermis. The cytoplasm looks empty. Elongated mitochondria are present close to the cell apex, and neurotubules extend vertically through the process. The cell surface shows only small microvilli, but it is provided with two to eight cilia with the common 9x2+2 pattern and a rootless centriole.

The outer pavillon epithelium is not as folded as the inner one; thus, its cells are of more constant cuboid shape and size (4-4.5 μ m). The microvilli covering the surface are about 1.7 μ m long. Numerous dark vesicles, mitochondria and RER are contained in the cytoplasm. The nucleus is close to the basal lamina. TEM reveals groups of two or three gland

cells in regular intervals. The dark cells closely resemble the secretory cells of the inner epithelium. All examined Gadilida possess an additional ring-fold distal and parallel to the supramarginal groove (Fig. 34).

Some of the gadilid species studied show subepithelial gland cells opening through the inner epithelium of the pavillon. The species are *Pulsellum lofotense* (M. Sars), *Wemersoniella turnerae* Scarabino, and all *Cadulus* species (Fig. 34) except *Cadulus* sp. (NMMH 803468) (Table 3).

DISCUSSION

FUNCTIONAL ASPECTS

The tubular nature of the peripodal pallial cavity can be considered a consequence of a rounding-off process of the mantle-shell-complex when adopting a burrowing habit (Pojeta and Runnegar, 1985; Runnegar and Pojeta, 1974; Runnegar, 1978; Salvini-Plawen, 1981, 1984). This process is recapitulated in scaphopod ontogeny (Lacaze-Duthiers, 1856-7; Kowalevsky, 1883). As in bivalves, the mantle cavity is not only the site of respiration, defecation and excretion, but also of food intake (Dinamani, 1964a, b). The organs of the mantle edges protect the animal against, and put it in touch with, its environment.

GLANDS

Experimental and detailed histochemical data on the functions of the various gland regions of the mantle epithelium

are lacking. Therefore, their functional interpretation must be based on structure and the observations on living animals alone.

The main function of the gland cells of the anterior mantle edge is probably mucus production. They closely resemble the mucus cells described in other marine invertebrates by Storch and Welsch (1972). This resemblance, the staining properties and the observations on living animals, indicate a mucoid nature of the secretions which have the same optical properties as water and can be discharged in copious amounts. Proteinaceous components may also be assumed, as the circum-nuclear cytoplasm is rich in rough endoplasmic reticulum. The basal portions of the cells are frequently associated with muscle fibers. Members of the order Dentaliida, where a prominent outer gland region is present, secrete enormous amounts of acid mucoid substances. The roles of the different types of gland cells are not clear. In *Antalis*, *Fustiaria* and *Rhabdus* the secretion is a sticky mass with the same optical properties as water. Gainey (1972) and Poon (1987) reported a feeding cavity in the sediment made by the foot after the animal is dug in. Gainey (1972) supposed that this cavity might be coated with mucus. At least for Dentaliida it is likely that this coating originates from the outer gland region of the anterior mantle edge. The weakly developed gland cells in the frontal epithelium of Gadilida are hardly capable of providing a mucus sheet to support a feeding cavity. In living *Entalina*, *Pulsellum* or *Cadulus* such massive mucus secretion has never

Table 3. Characters of the posterior mantle margin: (++) abundant; (+) present; (±) scarce, few; (−) absent; (?) no information.

	Aperture		Valve Mechanism								Pavillon		
	slit hori- zontal	slit verti- cal	ring sinus	dorsal flap	ventral bolster	subepith. gland cells	ciliated area	sphincter muscle	dorso- ventral con- strictors	ciliary organ	ciliated ledges	subepith. gland cells	addi- tional ring-fold
<i>Antalis</i>	+	−	+	+	+	+	+	+	−	−	−	±	−
<i>Dentalium</i>	+	−	+	+	+	+	+	+	−	−	−	+	−
<i>Fissidentalium</i>	+	−	+	+	+	+	+	+	−	−	−	±	−
<i>Graptacme</i>	+	−	+	+	+	+	+	+	−	−	−	±	−
<i>Fustiaria</i>	+	−	−	+	−	−	+	+	−	−	−	++	−
<i>Laevidentalium</i>	?	?	?	?	?	?	?	?	?	?	?	?	?
<i>Rhabdus</i>	+	−	+	+	+	+	+	+	−	−	−	±	−
<i>Entalina</i>	−	+	−	−	−	+	−	−	+	+	+	−	+
<i>Bathoxiphus</i>	−	+	−	−	−	±	−	−	+	+	+	−	+
<i>Heteroschismoides</i>	−	+	−	−	−	?	−	−	+	+	+	?	?
<i>Pulsellum</i>	−	+	−	−	−	+	−	−	+	+	+	±	+
<i>Annulipulsellum</i>	−	+	−	−	−	+	−	−	+	+	+	−	+
<i>Striopulsellum</i>	−	+	−	−	−	+	−	−	+	+	+	−	+
<i>Siphonodentalium</i>	−	+	−	−	−	+	−	−	+	+	+	−	+
<i>Wemersoniella</i>	−	+	−	−	−	±	−	−	+	+	+	++	+
<i>Gadila</i>	−	+	−	−	−	+	−	−	+	+	+	−	+
<i>Cadulus</i>	−	+	−	−	−	+	−	−	+	+	+	+	+

been observed.

Another function of the mucoid secretions of the anterior mantle edge appears to be the cleansing of the pallial opening from particles and the protection against disturbance by other organisms. The latter view is supported by the observation that moribund animals are invaded by protozoans, e.g. ciliates.

The inner gland region, common to all scaphopods, acts as a lubricator for the foot. This is not only important for locomotion and sealing of the pallial aperture but also for feeding. Gainey (1972) reported that the dentaliids *Antalis pseudohexagonum* Ihering (Henderson) and *Graptacme eboreum* (Conrad) collect food particles in the mantle cavity by ciliary action of the dorsomedian foot-furrow before they are ingested. The fact that gadilid scaphopods have epithelial as well as subepithelial gland cells in this region could point towards a compensation for the less elaborated outer gland region, compared to dentaliids.

The glandular region anterior to the preanal ciliary ridges has been regarded homologous with the hypobranchial gland of other molluscs by Distaso (1906). The absence of gills as a location reference and the lacking detailed structural analysis lend little weight to this hypothesis. However, it is likely that the mucoid secretion produced there lubricates fecal material and/or binds other particles in the mantle cavity before they are removed through one of its openings. It seems justified to assume that the subepithelial gland cells, which are most similar to those of the anterior mantle edge, secrete mucus for cleansing and repellent purposes.

Numerous authors (Simroth, 1894; Yonge, 1937; Stasek and McWilliams, 1973; Shimek, pers. comm.) ascribe the ability of shell reabsorption and truncation, as well as the formation of secondary apical shell features (pipes, plugs, lobes or slits) to the gland cells of the pavillon. Although this study provides no evidence for either of these functions, there are clear indications for the temporary nature of most apical shell features; they should therefore not be used as reliable systematic characters.

SENSORY RECEPTORS

The assumption that the receptor cells in the epithelia of the mantle margins have sensory qualities is based on their similarity with sensory cells of other invertebrates. The common characters are: an electron lucent cytoplasm of almost empty appearance, the presence of one or more cilia with or without sunken-in bases, neurotubules in parallel orientation to the cell axis, a long, subepithelial, dendritic process, the absence of nuclei in the epithelial region, and, if present, the collar of eight to nine stereomicrovilli. Chemo- and mechanosensory functions are ascribed to these types of ciliary receptor cells.

Sensory cells similar to type 1 and 1a of the anterior mantle edge of *Antalis* and *Fustiaria* have been described for

the siphons of the bivalves *Donax serra* Röding, *D. sordidus* Hanley and *Solen capensis* Fischer by Hodgson and Fielden (1984, 1986), as well as for *Schizochilus caecus* L'Hardy (Neorhabdocoela, Kalyptorhyncha) by Ehlers and Ehlers (1977). The collar-receptor of type 2 is of a very common type found in numerous metazoan taxa (Haszprunar, 1985; Salvini-Plawen, 1988).

Structures bearing certain similarity with the papillae of the frontal epithelium of *Entalina* and *Pulsellum* are reported for the tentacles of the bivalve *Placopecten magellanicus* (Gmelin) by Moir (1977), although in the latter the papillae exhibit collar-receptors with nuclei.

The ciliary organ of the dentaliid anterior mantle margin probably has no sensory qualities. The vividly beating cilia in living animals suggest the function of water current generation. The length of the cilia, compared to the pallial orifice diameter, is not sufficient to produce an effective flow of water in the mantle cavity unless the opening is maximally constricted. Alternatively, the ciliary organ could be responsible for water exchange over the frontal epithelium and thus facilitate chemoreception.

The dorsolateral slits in the anterior mantle edge of *Rhabdus rectius* probably represent true sensory organs. This remains to be confirmed by TEM investigations.

Contrary to the opinion of Distaso (1906), no osphradial sense organs are developed in the epithelium beneath the visceral ganglia. Distaso may have misinterpreted the pre-anal ciliary ridges and the medullary nature of the visceral connectives in this area.

No detailed descriptions of the sensory structures of the posterior mantle edge of Scaphopoda have been published. Reynolds (1988) gave a short report on the occurrence of multiciliated receptors in *Rhabdus rectius* (Carpenter), but no comparison with the corresponding structures in *Entalina quinquangularis* (Forbes) can be made at present.

APICAL VALVE-ORGAN

ANTALIS - TYPE

The arrangement of muscles and connective tissue in *Antalis* spp. indicates that the width of the opening is regulated by the dorsal section of the annular fold, while the ventral part acts as an abutment for the musculature. The dorsal section closes the aperture when it is pressed against the ventral part and against the semi-annual projection on the dorsal side. The action of the sphincter alone is probably not responsible for this; blood pressure in the sinus certainly helps to close the valve. The muscle fibers transversing the dorsal section of the sinus open the valve.

FUSTIARIA - TYPE

Due to the lack of a ventral bolster and a sphincter muscle, rising blood pressure is likely to be the only force

lowering the large dorsal flap. Like in the *Antalis*-type, decreasing pressure and contraction of the dorsoventral muscle fibers lift the flap to open the pallial orifice.

ENTALINA - TYPE

The closure of the mantle opening to a vertical slit is probably effected by the dorsoventral muscle bundles on either side of the orifice. Dorsally and ventrally attached to connective tissue sockets, they constrict the orifice by approximating its lateral walls. Increasing haemolymph pressure in the surrounding lacunae is probably synergetic. The antagonistic force is provided by the radially arranged muscle fibers between inner and outer mantle epithelia.

WATER CIRCULATION

Respiration, defecation and excretion depend on the currents of water through the mantle cavity. Its surface has to meet the respiratory demands of the organism, as ctenidia are not present. Yonge (1937) gave a detailed account of the currents in the pallial cavity of *Antalis entalis*. He described the apical orifice as the inhalent and exhalent opening, observing a steady inflow of water due to ciliary action, followed by a violent expulsion, which is caused by a sudden retraction of the foot. In general, these results can be confirmed, although some complementary remarks can be made.

The inhalent water currents are produced by the ciliary organ of the pavillon and the preanal ridges in Gadilida, while in Denticulida the ciliary beat of the more numerous preanal ridges alone generate the flow. The metachronal waves of the cilia can be easily observed through the transparent shells of *Rhabdus*, *Pulsellum* and *Cadulus*. Contrary to Yonge's reports, the apically entering water leaves the mantle cavity through the anterior aperture. This was observed in *Entalina quinquangularis*, *Pulsellum lofotense*, *Cadulus subfusiformis*, and *Antalis occidentalis* (Stimpson). Suspended carmine particles and sperm could be followed through the mantle cavity.

During the flow of water through the pallial cavity, the foot can only play a minor role in producing the inhalent current. The piston-like actions of the foot causing the rapid apical exhalent current occur about twice a minute in relaxed specimens of *Antalis* and *Fustiaria* (Denticulida). The gadilid species showed intervals of one minute or more between expulsions. During the expulsions and during burrowing action the ciliary beat ceases; it is resumed a few seconds later.

SYSTEMATICS IMPLICATIONS

Systematics below the order level are solely based on shell and radula characters. Chistikov (1975a, b) attempted to include some criteria of the soft body.

The results of the present study provide detailed information on the differences of the mantle edges between the

scaphopod orders, but also allow some statements at the family level.

DENTALIIDA

The anterior pallial edge of the order Denticulida is characterized by a conspicuous outer gland region, a strong ring of cartilage-like material, as well as massive fibrous connective tissue and muscles in the central fold, a circular ciliary organ, and an inner gland region with epithelial gland cells only. The average number of preanal ciliary ridges ranges from 12 to 15.

In most representatives of this order the valve organ of the posterior mantle edge shows a dorsal and a ventral elevation of the pallial wall in combination with an annular blood sinus. Closing of the horizontal slit is achieved by lowering the dorsal flap by means of muscular action and haemolymph pressure. No ciliary tracts are developed on the ledges of the pavillon proper.

These features are shared by most of the examined denticulid genera, such as *Antalis*, *Dentalium*, *Fissidentalium* and *Laevidentalium*. Thus, analysis of this organ system would suggest the grouping of these genera in the family Denticulidae. With the exception of *Laevidentalium*, this grouping agrees with that of Scarabino (1979).

The genus *Fustiaria* differs from the general scheme in three aspects: 1) The radially arranged fibers of connective tissue in the central fold of the anterior mantle edge are not developed; 2) The relative abundances of the two types of sensory cells are completely different, and an additional kind of ciliary receptor is present (type Ia); 3) The most complex difference to the *Antalis*-type organization concerns the posterior mantle edge.

These differences, together with the particularities of the shell, the radula (Scarabino, 1979), and the foot (Steiner, in press), justify the separation of *Fustiaria* from the Denticulidae. Thus, a new monogeneric family, the Fustiariidae, is proposed.

FUSTIARIIDAE (FAM. NOV.)

DIAGNOSIS — Denticulid Scaphopoda with smooth, polished, thin-walled and transparent shells of slender shape, moderate curvature and circular cross-section. The ventral side of the apex can have a straight, deep slit or a small notch. The rachis tooth of the radula differs from that of Denticulidae by showing a flat superior edge. The ciliary organ of the anterior pallial margin consists of five to six rows of cells. The posterior mantle orifice can be closed by a dorsal flap. No ventral bolster, sphincter muscle or glands are elaborated there. Instead, subepithelial gland cells are abundant in the pavillon. The pedal sinus is divided by a horizontal septum.

TYPE GENUS — *Fustiaria* Stoliczka, 1868. The genus *Rhabdus*, represented by *R. rectius* in this study, has a

remarkable specialization at the anterior mantle margin. A pair of dorso-lateral invaginations at the periphery of the outer gland region are developed, and the ciliary organ is wanting. The slit-like invaginations can either be considered denovo differentiations of the outer gland replacing the lost ciliary organ, or a differentiation of the ciliary organ itself.

The family Rhabdidae was erected by Chistikov (1975a), who emphasized the peculiar morphology of radula and shell. The special elaboration of the anterior pallial edge supports the separation from the other dentaliid Scaphopoda.

GADILIDA

The anterior mantle edge of Gadilida differs from the corresponding organ of Dentaliida by the lack of an outer gland region and the ciliary organ. The frontal epithelium is elaborated into papillae which carry ciliary sensory cells. The amount of cartilage-like and fibrous connective tissue in the central fold varies species-specifically. The inner gland region exhibits subepithelial as well as epithelial gland cells. The average number of ciliary rings in the preanal area (4 to 8) is lower than in the Dentaliida.

The posterior pallial edge is characterized by a powerful ciliary organ of large ciliated cells arranged in a single row encircling the mantle opening. Dorsoventral muscle fibers close the aperture by approximating the lateral walls of the pallial opening, thus producing a vertical slit. No distinct annular blood sinus is developed. The ledges of the pavillon proper are lined by ciliary tracts.

At present the only anatomical character suitable for a classification within the Gadilida is the different abundances of papillae in the frontal epithelium of the anterior mantle edge. These structures were found in great numbers in the examined species of *Entalina*, *Bathoxiphus*, *Heteroschismoides*, *Pulsellum*, *Annulipulsellum*, *Striopulsellum* and *Wemersoniella*. Comparatively few papillae are developed in *Siphonodentalium*, *Cadulus* and *Gadila*.

For the common ancestor of Dentaliida and Gadilida the following differentiations of the mantle can be assumed: 1) a rigid ring formed by connective tissue and muscles to support the anterior pallial opening, 2) an area of epithelial gland cells inside the mantle cavity (= inner gland region), 3) several ciliated, annular ridges in the preanal region, perhaps preceded by a ventral glandular field (=hypobranchial gland of Distaso), 4) the posterior mantle opening with an elaborated dorsal extension, the pavillon proper.

According to this list, the outer gland region and the presence of a ciliated organ at the anterior pallial aperture are differentiations within the Dentaliida, while the subepithelial gland cells of the inner gland region and the ciliation of the pavillon proper have developed in Gadilida only.

ACKNOWLEDGMENTS

I thank V. Scarabino and B. Metivier (Mus. Hist. Nat., Paris), B. Marshall (Nat. Mus., New Zealand), J. Knudsen (Zoologisk Museum, Copenhagen), G. Davies (University of Edinburgh) and R. Houbbrick and D. Bohmhauser (Nat. Mus. Nat. Hist., Washington, D.C.) for generously loaning scaphopod specimens. Collection of living Scaphopoda was financially supported by travel-grants of the Bundesministerium für Wissenschaft und Forschung and by a grant-in-aid of Sigma Xi, The Scientific Research Society. I am indebted to my supervisor, Professor L. Salvini-Plawen, for his help and for the critical reading of the manuscript.

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Date of manuscript acceptance: 28 April 1991

The freshwater mussels (Bivalvia: Unionoidea) of the upper Delaware River drainage

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Abstract. We surveyed the unionoidean fauna of 44 sites in the upper Delaware River drainage of New York during 1990. Seven species of unionoideans were found living in the basin, including the endangered *Alasmidonta heterodon* (Lea, 1829). Four other species are known historically from the upper Delaware basin, but now are either absent from the area or restricted to a few local sites. Neither calcium concentration nor stream size is a good predictor of unionoidean species richness in the study area. We hypothesize that sediment stability could regulate the occurrence of unionoideans in the streams of the upper Delaware drainage.

The Delaware River is one of the major rivers of the northern Atlantic Slope. Ortmann (1919) showed that the lower Delaware basin contained a rich Atlantic Slope fauna, including the endangered species *Alasmidonta heterodon* (Lea, 1829) and the southernmost known population of *Margaritifera margaritifera* (Linnaeus, 1758). Because the upper Delaware is known for its high water quality, we felt that similarly rich communities of unionoideans could live upstream of the area surveyed by Ortmann. There is little published information on the unionoideans of the upper Delaware basin. Marshall (1895) reported six species from unspecified sites in the Delaware River system in New York. Harman (1975) published a brief article focusing on the effects of anthropogenic disturbances on the molluscan community of the Delaware's headwaters. We surveyed the waters of the upper Delaware drainage in New York in 1990 to determine whether *A. heterodon* lived in this area and to assess the current status of the freshwater mussel community in general.

THE STUDY AREA

Our survey covered the streams in the Delaware River basin in New York (Fig. 1). Streams range in size from headwater brooks to the Delaware River itself, which has a mean annual discharge of 160 m³/sec at Port Jervis (our station 1) (Zembrzuski *et al.*, 1983). Most streams in the study area have fairly high gradients, and sediments consist chiefly of cobbles, gravel, and coarse sand. The water in most streams is very clear and somewhat soft (Table 1).

Most of the watershed is forested, although there is some agriculture, and villages and small cities are scattered along the Delaware River and its major tributaries. The largest

municipalities in the basin are Port Jervis (pop. 8699), Monticello (6306), and Hancock (1526), so urban pollution is not pronounced. The larger streams in the upper Delaware basin are used heavily for recreation (boating, fishing). The major current anthropogenic impacts on the streams in the drainage probably arise from the three large reservoirs of the New York City water supply system. These reservoirs alter the hydrological and thermal characteristics (all three reservoirs are hypolimnetic release) of downstream waters (the lower East and West Branches of the Delaware River, the upper mainstem of the Delaware River, and the middle Neversink River). About 30 m³/sec of water is diverted out of the basin from these reservoirs to supply drinking water for New York City (Zembrzuski *et al.*, 1983).

METHODS

We visited 44 sites on the upper Delaware River drainage during periods of low, clear water between July and September, 1990, collecting mussels by handpicking while wading or snorkeling. Most specimens were identified and returned immediately to the stream. Voucher specimens (chiefly dead shells) have been deposited in the New York State Museum (NYSM) and Academy of Natural Sciences at Philadelphia (ANSP). In addition to our field collections, we searched the collections of the National Museum of Natural History (USNM), American Museum of Natural History (AMNH), and NYSM for specimens of unionoideans from the upper Delaware basin. Mussel nomenclature follows that of Turgeon *et al.* (1988). Water samples were collected in clean polyethylene bottles and analyzed for calcium by plasma emission using a Perkin-Elmer ICP/6000.



Fig. 1. Location of the Delaware River basin and sampling sites on the upper Delaware River basin. Inset shows the Delaware (D) and nearby drainages (H = Hudson, S = Susquehanna) in New York, New Jersey, and Pennsylvania; the study area is stippled. Site numbers on the main figure correspond to those given in Table 1. Open circles show sites where unionoideans were not found, small black circles show sites where only *Elliptio complanata* was found, and large black circles show sites where at least two species of unionaceans were found. Dotted lines are county boundaries.

RESULTS AND DISCUSSION

The waters of the upper Delaware River drainage now support seven species of unionoideans (Table 1). Four other species are known only through historical records. Marshall (1895) reported *Ligumia nasuta* (Say, 1817), *Lampsilis cariosa* (Say, 1817), and *L. radiata* (Gmelin, 1791) from the upper Delaware system, but we saw no trace of these species in 1990 [Marshall's report of *Anodontoides ferussacianus* (Lea, 1834) probably is based on a misidentification of an *Anodonta* sp.]. *Margaritifera margaritifera* is represented by a single shell (AMNH 164659) taken from "lake at Camp Welmet near Narrowsburg, Sullivan Co., NY" by H. S. Feinberg in 1949. Unfortunately, we were unable to get access to the lake at Camp Welmet (Silver Lake, not Lake Welmet, which, confusingly enough, is not on the property of Camp Welmet) in 1990 to assess the status of this population.

Of the seven species still living in the upper Delaware basin, *Elliptio complanata* is by far the most abundant and widespread. In fact, we found *E. complanata* at every site where unionoideans were present. Although many authors have commented on the broad ecological tolerances of this species (e.g. Ortmann, 1919; Clarke and Berg, 1959; Strayer, 1987), we know surprisingly little about what behavioral, physiological, or ecological adaptations allow this species to succeed over such a broad range of habitats.

There are old, indefinite reports of *Alasmidonta*

heterodon from New York (Marshall, 1895; Letson, 1905), but ours are the first reliable records of this species from New York (there are no museum lots of this species from New York in the NYSM, AMNH, USNM, ANSP, University of Michigan Museum of Zoology, or Museum of Comparative Zoology collections.) There is apparently a healthy population of this species in the lower 12-18 km of the Neversink River. Our findings raise the obvious possibility that *A. heterodon* could still persist in other tributaries of the upper Delaware (or in the river itself) in New Jersey or Pennsylvania.

The other two species of *Alasmidonta* (*A. undulata* and *A. varicosa*) also were abundant in the Neversink River drainage. In addition, we found *A. undulata* in the West Branch of the Delaware River above Cannonsville Reservoir. It is possible that small numbers of *A. varicosa* may live in the upper West Branch as well, although we did not find it there in 1990. Harman (1975) reported *A. marginata* Say, 1818, a species that resembles *A. varicosa*, but which probably does not occur in the Delaware basin, in the upper West Branch.

Anodonta implicata is found in small numbers in the lower Neversink River near Port Jervis. *A. implicata* is parasitic on anadromous shad and herring (*Alosa* spp.), and is found typically in low-gradient coastal rivers and ponds (Johnson, 1946; Davenport and Warmuth, 1965; Smith, 1985; Strayer, 1987). Our records from the upper Delaware River system are interesting for two reasons. First, the reach of the Neversink River occupied by *A. implicata* is a relatively high-gradient, stony, upland river, unlike the coastal sites typically frequented by this species. Second, although large numbers of American shad [*Alosa sapidissima* (Wilson, 1811)] run upstream to well above the junction of the East and West Branches of the Delaware, we found no trace of *A. implicata* in most of the mainstem, even in such apparently suitable habitat as the huge, quiet pool at Narrowsburg. This observation suggests that some ecological factor other than the distribution of the host fish determines the current distribution of *A. implicata* in the Delaware system.

One of the most striking impressions from our work was just how poor the unionoidean communities were over large parts of the upper Delaware basin. Unionoideans apparently were absent at many (30%) of the sites that we surveyed, even though the streams were large enough to support unionoideans and were not obviously polluted. In some of these cases (e.g. stations 30, 33), the sediments consisted mainly of well rounded cobbles, and probably are too coarse and too unstable for unionoideans. Other sites (e.g. stations 18-20) apparently have suitable substrata, high water quality, and diverse fish communities, but no trace of unionoideans. We do not know what is keeping unionoideans out of these sites. One obvious possibility that we believe we can rule out is inadequate dissolved calcium. Although many waters in the basin are soft, there is no relationship between calcium con-

Table 1. Distribution of unionid bivalves in the upper Delaware River basin in 1990 (numbers show numbers of living animals collected; d, old, dead shells found; D, recently dead shells found).

	calcium concentration (mg/l)	man-hours searching	<i>Elliptio complanatus</i> (Lightfoot, 1786)	<i>Alasmodonta heterodon</i> (Lea, 1829)	<i>A. undulata</i> (Say, 1817)	<i>A. varicosa</i> (Lamarck, 1819)	<i>Anodonta cataraeta</i> Say, 1817	<i>A. imbecilis</i> Say, 1829	<i>Syrphites undulatus</i> (Say, 1817)
1. Delaware River, Port Jervis	8.7	1.5	2					d	
2. Delaware River, Mongaup		1.2	28						
3. Delaware River, Pond Eddy		1	26						
4. Delaware River at Roebling Bridge near Minisink	7.5	0.5	D						
5. Delaware River, Narrowsburg	7.2	2.2	d						
6. Delaware River, Skinner's Falls		1	2						
7. Delaware River, Stalker (PA)		1	D						
8. Delaware River, Lordsville	7.7	0.5	d						
9. Neversink River, Port Jervis		2.2	4						
10. Neversink River, Huguenot	7.8	3	128	7	2	1		2	2
11. Neversink River, Graham Road		6	64	10	1	20		3	5
12. Neversink River, Roses Point		6	52	7	3	76		1	26
13. Neversink River, Oakland Valley	5.8	1.5	1						
14. Neversink River, Bridgeville	5.6	1.5	D		1				
15. Neversink River at mouth of Sheldrake Stream		2.5	57		51	2			
16. Neversink River, Ranch Hill Road		2	2		11	1			
17. Neversink River, Woodbourne	3.9	1.5	D						
18. Basher Kill, Galley Hill Road		0.7							
19. Basher Kill, Westbrookville		1							
20. Basher Kill, Wurtsboro	7.4	0.8							
21. Delaware and Hudson Canal, Bova Road		0.7	5						
22. Sheldrake Stream at mouth	7.8	1.3	41		18		1		
23. Sheldrake Stream, Thompsonville		1	2						
24. Sheldrake Stream, Ranch Hill Road		1	9		D		D		
25. Mongaup River, Route 97		0.3							
26. Mongaup River south of Swinging Bridge Reservoir	5.9	0.8							
27. West Branch Mongaup River, Gale Road	7.9	0.5							
28. Ten Mile River below Route 97		0.2							
29. East Branch Ten Mile River, County Rte. 23	3.6	0.5	2						
30. Callicoon Creek at mouth	9.2	0.5							
31. Callicoon Creek, Hortonville		0.5							
32. East Branch Callicoon Creek below Route 52	8.2	0.5							
33. North Branch Callicoon Creek 2 miles above Hortonville	9.6	0.2							
34. East Branch Delaware River, Peas Eddy	7.5	1	60						
35. East Branch Delaware River, Fish's Eddy		1.5	11						2
36. East Branch Delaware River, Downsville	5.8	0.5							
37. East Branch Delaware River, Margaretville	8.7	0.5							
38. West Branch Delaware River, Hancock		1	D						
39. West Branch Delaware River, Hale Eddy	6.8	1	1						

Table 1. (continued)

	calcium concentration (mg/l)	man-hours searching	<i>Elliptio complanata</i> (Lightfoot, 1786)	<i>Alasmidonta heterodon</i> (Lea, 1829)	<i>A. undulata</i> (Say, 1817)	<i>A. varicosa</i> (Lamarck, 1819)	<i>Anodonta cataracta</i> Say, 1817	<i>A. implicata</i> Say, 1829	<i>Strophitus undulatus</i> (Say, 1817)
40. West Branch Delaware River, Deposit		0.5	d						
41. West Branch Delaware River, Walton	11.5	1	D						
42. West Branch Delaware River, Hamden		2.5	2		1				1
43. West Branch Delaware River, Delhi	14.9	2	55		12				3
44. West Branch Delaware River, Bloomville	14.9	1	d		D				

centration and either unionoidean density or species richness (Fig. 2). Harman (1975) believed that the operation of the New York City water supply reservoirs eliminated most unionoideans from tailwater reaches, but our richest sites were downstream of such a reservoir on the Neversink River.

A second piece of evidence that shows the poverty of the upper Delaware River unionoidean community is that 58% of the sites that contain unionoideans contain only one species, *Elliptio complanata*. The dominance of *E. complanata* is especially striking in the main Delaware River, where we found only a single, old shell of *Anodonta implicata* along with more than 500 living or recently dead specimens of *E. complanata*. The main Delaware is a large river with a rich fish fauna, and would be expected to support several (6-10) species of unionoideans, as was the case on the Susquehanna and lower Delaware rivers (Ortmann, 1919; Clarke and Berg, 1959; Harman, 1970). Clarke (1986) recently found that parts of the upper Connecticut River that formerly supported several species of unionoideans now con-

tain only *E. complanata*. He suggested that the operation of hydroelectric dams could have eliminated most of the unionoidean species without, however, suggesting why *E. complanata* would be resistant to these recent environmental changes. Marshall's (1895) old records of species such as *Lampsilis cariosa* from the basin suggest that some unionoidean species could have likewise been extirpated from the mainstem Delaware River. It is possible that past episodes of pollution, perhaps from wood processing industries in the Delaware basin (Myers, 1986), could have destroyed the unionid fauna in some streams, but it is unclear why only *E. complanata* would be able to recolonize these reaches once the pollution stopped.

Finally, unionoideans are highly localized even in sites where several species are present (e.g. stations 10-12). At these sites, there often are sharp boundaries between dense (> 1 individual/m²), multispecific beds of mussels and areas entirely devoid of mussels. These sharp boundaries do not generally correspond to obvious changes in environmental conditions (e.g. sediment grain size, current velocity, water depth), although a detailed, quantitative study like that of Salmon and Green (1983) probably would uncover statistically significant differences in environmental conditions between mussel beds and nearby areas devoid of mussels. An alternative hypothesis is that the mussel beds represent areas of relatively stable sediments. It is well known (e.g. Leopold *et al.*, 1964; Richards, 1982) that most stream sediments are set in motion by floods every year or two. The instability of sediments poses obvious problems for the long-lived Unionoidea, which could be displaced, crushed, or buried when the sediments in which they live are moved. Vannote and Minshall (1982) showed that sediment stability was a major factor regulating the local distribution of mussels in the Salmon River canyon, Idaho. We suggest that sediment stability is generally important to mussels in streams, and that the

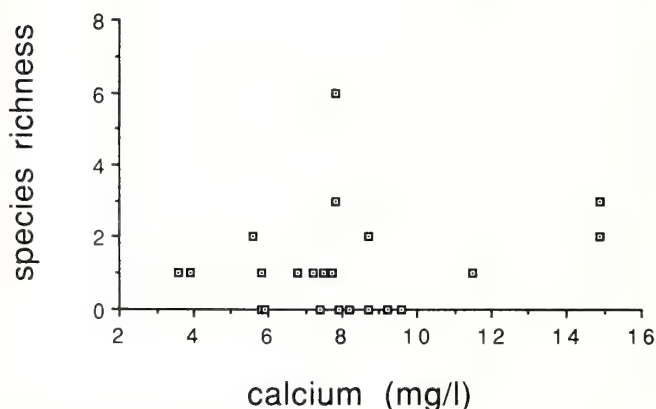


Fig. 2. Species richness of unionoideans in streams of the upper Delaware basin as a function of calcium concentrations ($r = 0.22$, NS).

highly local mussel beds that we observed in the Neversink and elsewhere represent not particularly favorable conditions of sediment grain size, current velocity, and so on, but rather areas in which the sediments have not been moved for some time (a decade or so?), or are stable during critical periods such as during recruitment of juveniles.

We have devoted some space to this speculative discussion of potential controlling factors because we feel that, despite a large volume of research on unionoidean ecology, there is little real understanding of what controls unionoidean distribution and abundance in streams. Why does the lower Neversink River contain a rich community of unionoideans, including an endangered species, while other apparently suitable sites nearby support only one species or no unionoideans at all? Until we can answer questions like these, it will be difficult to formulate intelligent management schemes to protect our remaining unionoidean communities.

ACKNOWLEDGMENTS

We are grateful to the USNM, AMNH, NYSM, Paul Greenhall, Walter Sage, and Norton Miller for allowing us to examine their collections of unionoideans, to Ed Blakemore for help in depositing voucher specimens, and to Kathy Schneider and Paul Novak for help in the field. Kathy Schneider also provided encouragement and logistical support. This work was funded by the United States Fish and Wildlife Service through The New York Natural Heritage Program of the Department of Environmental Conservation, with additional support from a grant to the Institute of Ecosystem Studies from the General Reinsurance Corporation. This is a contribution to the program of the Institute of Ecosystem Studies of The New York Botanical Garden.

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Date of manuscript acceptance: 27 March 1991

The Naiades (Bivalvia: Unionoidea) of the Delmarva Peninsula

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Abstract. A survey comprising 307 stations of the freshwaters of the Delmarva Peninsula of Delaware, Maryland, and Virginia was conducted to determine the species diversity and zoogeographic distribution of unionid bivalves. The unionid fauna included *Elliptio fisheriana* (Lea, 1838), *E. complanata* (Lightfoot, 1786), *Lampsilis radiata* (Gmelin, 1791), *Leptodea ochracea* (Say, 1817), *Anodonta cataracta* Say, 1817, *A. implicata* Say, 1829, *Ligumia nasuta* (Say, 1817), *Strophitus undulatus* (Say, 1817), *Alasmidonta undulata* (Say, 1817), and the rare and endangered *A. heterodon* (Lea, 1830). Although a review of the literature and a survey of museum collections revealed records for *E. dilatata* (Rafinesque, 1820), *L. cariosa* (Say, 1817) and *A. varicosa* (Lamarck, 1819), these species were not found during the field survey. No populations of freshwater unionids were found south of the Maryland/Virginia state line. Comparisons of collections in this study with those reported in the literature and in museums indicate a general decline in diversity. The federally listed rare and endangered *A. heterodon* is reported from the Choptank drainage in Maryland. No populations of the zebra mussel, *Dreissena polymorpha* (Pallas, 1771) were found.

Rhoads (1904), in a brief examination of the molluscan fauna of Delaware, remarked that the literature on the molluscan fauna of Delaware was practically nonexistent. This was true in 1904 and is still true nearly 80 years later, not only for Delaware but for the entire Delmarva Peninsula. Rhoads (1904), after collecting at three localities, reported the presence of ten unionid species: *Lampsilis nasutus* (Say, 1817) [= *Ligumia nasuta* (Say, 1817)], *L. radiatus* (Gmelin, 1791) [= *L. radiata* (Gmelin, 1791)], *L. cariosus* (Say, 1817) [= *L. cariosa* (Say, 1817)], *L. ochraceus* (Say, 1817) [= *L. ochracea* (Say, 1817)], *Unio complanatus* ('Solander' Dillwyn, 1817) [= *Elliptio complanata* (Lightfoot, 1786)], *U. fisherianus* Lea, 1838 [= *E. fisheriana* (Lea, 1838)], *Anodonta cataracta* Say, 1817, *Strophitus edentulus* (Say, 1829) [= *S. undulata* (Say, 1817)], *S. undulatus*, and *Alasmidonta marginata varicosa* (Lamarck, 1819) [= *A. varicosa* (Lamarck, 1819)]. This list remains the most complete faunal record for the naiades of the entire Delmarva peninsula.

Since the report of Rhoads, several other works have mentioned the unionid fauna of the peninsula only in passing (Ortmann, 1919; Johnson, 1970; Davis and Fuller, 1981; Davis *et al.*, 1981; Clarke, 1981; Davis, 1984). Ortmann (1919) mentioned *Elliptio fisheriana* from the Chester River system of Maryland. Johnson (1970), in his treatment of the freshwater bivalves of the Atlantic Slope, documented only the unionid species inhabiting the waters draining the western

shore of Chesapeake Bay. He (Johnson, 1970) made reference to only *E. fisheriana* (Lea, 1838), which he considered a junior synonym of *E. lanceolata*, as having its type locality at the head of the Chester River in Kent Co., Maryland. Davis and Fuller (1981) reported *Lampsilis radiata* (Gmelin, 1791) from Sussex Co., Delaware, without more precise locality data. Davis (1984) reported *E. fisheriana* and *E. dilatata* (Rafinesque, 1819) from Concord Pond, Sussex Co., Delaware, *E. complanata* from Deep Creek, Sussex Co., Delaware, and the Sassafras River, Kent Co., and Chester River, Queen Anne's Co., Maryland. Clarke (1981) noted the presence of *Alasmidonta undulata* at "Choptank Mills," Kent Co., and *A. varicosa* at the head of Red Clay Creek, New Castle Co., Delaware.

These few reports constitute the published information available on the freshwater naiades of the Delmarva Peninsula. Given this paucity of information, the potential threat of exotic species introductions, e.g. *Dreissena polymorpha* (Pallas, 1771) (Counts *et al.*, 1991; Handwerker and Counts, 1991), and the geographic features unique to the peninsula, the present study was undertaken to provide a recent baseline study of species diversity and zoogeographic distribution of the freshwater unionids indigenous to the Delmarva Peninsula. The distribution of the Asian clam, *Corbicula fluminea* (Müller, 1774) on the Delmarva Peninsula is discussed in Handwerker *et al.* (1991).

DESCRIPTION OF THE STUDY AREA

The Delmarva Peninsula, as defined here, extends from Cecil Co., Maryland, east of the Susquehanna River, and New Castle Co., Delaware, and includes all that land lying between the Susquehanna River and Chesapeake Bay on the west and the Delaware River and Bay and Atlantic Ocean on the east, south to Cape Charles, Virginia. The peninsula is bisected on an east-west axis by the brackish waters of the Chesapeake-Delaware Canal. Hence, the lower portion of the peninsula is an island. The study area included the entire state of Delaware, the nine Eastern Shore counties of Maryland (from Cecil Co. in the north to Somerset - Worcester counties in the south) and Virginia (Accomack and Northampton counties). Geologically, the Delmarva Peninsula is composed of deposits ranging from the Late Cretaceous Potomac Group in the north to the Pleistocene sands of the southern half of the peninsula (Stephenson *et al.*, 1933).

The freshwaters of the Delmarva Peninsula drain into the Chesapeake Bay on the west or into the Delaware Bay or Atlantic Ocean on the east. River systems draining into Chesapeake Bay are generally longer and wide than those of the eastern portions of the peninsula that drain into the Atlantic Ocean, either directly or via the Delaware River and Bay. Regardless of the drainage, the streams of the peninsula are tidal and saline for major portions of their length. The transition from brackish to freshwater is usually abrupt and occurs at mill dams. In some cases (e.g. the Sassafras River) the tidal, brackish portion of the stream is substantially longer than the freshwater. The freshwater drainages of the Maryland portion of the peninsula have been described by Carpenter (1983). Generally, streams flow at a slow rate ($0.25 - 3.74 \text{ m}^3/\text{sec}$) and have a maximum discharge rate ranging between $30.0 - 212.25 \text{ m}^3/\text{sec}$ and minimum discharge rates ranging between $0 - 0.37 \text{ m}^3/\text{sec}$ (Carpenter, 1983).

All ponds of the Delmarva Peninsula were originally impounded to store water for livestock and to power grain mills or, in the case of those on the upper peninsula, manufacturing facilities. While none of the mills are now operational, the ponds have been preserved and many are maintained as recreational areas.

The Delmarva Peninsula is also the northern-most point at which cypress swamps occur on the Atlantic coast. Many of the streams of southern Delaware and Somerset, Wicomico, and Worcester counties, Maryland (e.g. Nanticoke River, Pocomoke River, Dividing Creek), drain these swamps and these streams are typical "blackwater" systems.

Many of the freshwater streams of the peninsula are channelized and characterized by steep banks that lack vegetation other than grasses. These streams, and their headwaters, serve to drain cultivated fields. Because of the intense agricultural development of the peninsula, freshwaters show a high degree of eutrophication south of the Chesapeake-Delaware Canal. Most of the stream systems in this

agricultural region are little more than drainage ditches between cultivated fields, particularly those of the Virginia counties.

METHODS

A review of the literature (Rhoads, 1904; Ortmann, 1919; Clarke, 1981; Davis and Fuller, 1981; Davis, 1984) was conducted in conjunction with a survey of the unionid collections of the Academy of Natural Sciences of Philadelphia (ANSP) and Delaware Museum of Natural History (DMNH) for collections made on the Delmarva Peninsula. This museum survey was conducted to verify published records and to record and consolidate any unpublished records reflected by these collections. These records were collated and localities listed were then surveyed for the presence of species historically reported or collected.

A survey of major drainage systems of the Delmarva Peninsula (Appendix, Fig. 1) was conducted from August 1989 through August 1990. The survey included 307 stations. A description of all stations surveyed is on file at the Academy of Natural Sciences of Philadelphia. Stations were defined as the section of the stream 100 m above and below the point

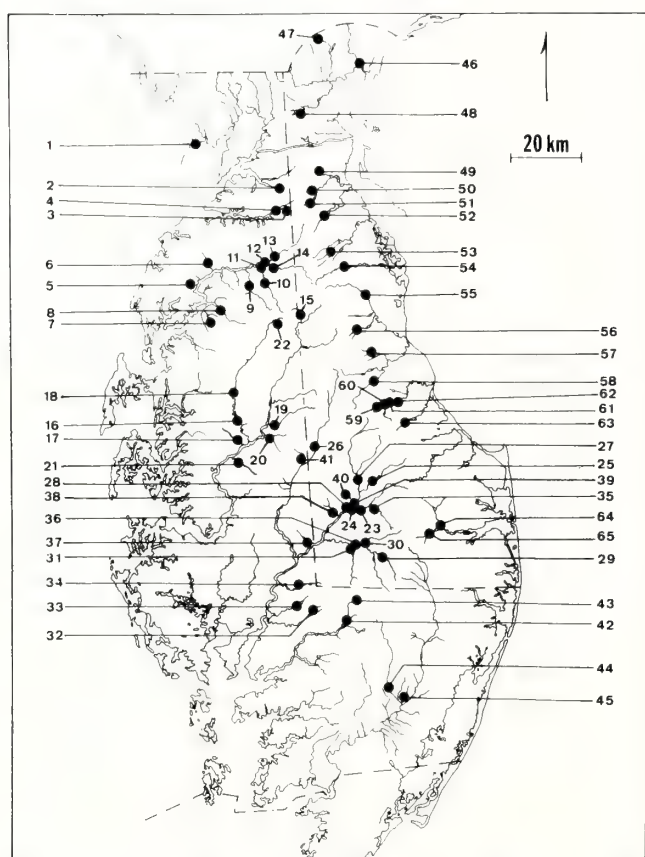


Fig. 1. Station locations at which naiades were collected in the northern half of the Delmarva Peninsula (Delaware and Maryland).

at which the stream was entered. Stream surveys included tidal and brackish water portions of streams where these conditions occurred, along the Chesapeake Bay and the Delaware River estuary. Bivalves were collected by hand and small dredge and by screening substrata. Representative specimens were placed in the collections held at the Coastal Ecology Research Laboratory at the University of Maryland Eastern Shore (UMES).

Collections made at sites reported in the literature or which were represented in the ANSP and DMNH were compared to determine changes in species diversity. All localities reported from those sources were surveyed when locality data were sufficient to relocate the original site.

RESULTS

Ten species of unionids were found in the waters of the Delmarva Peninsula. Historic records were found for three additional species. Station numbers from the present study are given in parentheses for surveys conducted at localities listed in published works and the museum records. Station localities are provided in the appendix.

Elliptio fisheriana (Lea, 1838)

Published Records. Chester River System: Head of the Chester River (Kent Co.) MD [Type Locality (Lea, 1838)]; Stations 5 (Ortmann, 1919), 8 (Ortmann, 1919), 15 (Rhoads, 1904), 23 (Davis, 1984), 28 (Rhoads, 1904).

Museum Materials Examined. Stations 5, 13, 17, 18, 23, 24, 28. Also examined were specimens from an unnamed body of water, Chestertown, Kent Co., MD (DMNH 174253) and from Sussex Co., DE (ANSP 345052).

Records from Present Study. Stations 11, 17, 29, 30, 31, 34, 35 - 39.

There has been confusion as to the systematic position of *Elliptio fisheriana*. Ortmann (1919) noted that a close morphological similarity exists between *E. fisheriana* and *E. cupreus* and believed that *E. fisheriana* could be a lowland race of *E. cupreus*. However, he also noted that he could not detect intergrades between the two species. He further noted that specimens collected in White Clay Creek, Chester Co., Pennsylvania (Hartman and Michener, 1874), could in fact have been collected in Delaware. Johnson (1970) reported *E. fisheriana* to be a synonym of *E. lanceolata* (Lea, 1828) which he considered to be a highly variable species (25 synonyms listed for *E. lanceolata*); however, Davis (1984) found *E. fisheriana* to be genetically distinct from *E. lanceolata*. No published reports exist regarding the glochidia, soft tissue anatomy, or breeding season of *E. fisheriana*.

Elliptio complanata (Lightfoot, 1786)

Published Records. Stations 4 (Davis, 1984), 24 (Davis,

1984). An additional record was published for the Chester River, Queen Annes Co., MD (Davis, 1984).

Museum Materials Examined. Stations 3, 4, 12 - 14, 17, 18, 21, 22 - 24, 28, 43, 46. Additional specimens: Elk River, Sandy Cove, Cecil Co., MD (DMNH 75226); an unnamed ditch, Petersburg, Kent Co., DE, No Date, (DMNH 131124); Christmas Creek, Newark, New Castle Co., DE (ANSP 366090); Small Creek, Kent Co., DE (ANSP 358279).

Records from Present Study. Stations 1, 6, 9, 10, 12, 13, 17 - 19, 21, 22, 25 - 27, 30 - 32, 34, 37, 48, 49, 53, 54, 59, 61, 63.

Elliptio complanata can demonstrate local morphological variability in both shell shape and coloration. Further, the species has the widest zoogeographic distribution of those unionid bivalves indigenous to the Atlantic Slope, ranging from the Apalachicola River drainage of Florida to the St. Lawrence drainage and Interior Basin of Canada (Burch, 1973; Clarke, 1973). Perhaps it is because of this wide variability and zoogeographic distribution that numerous synonyms exist for the species [124 in Johnson (1970)]. Soft tissue anatomy has been described by Ortmann (1911) and Reardon (1929).

Elliptio complanata is reported to breed from April through July or August. The host species is the anadromous yellow perch, *Perca flavescens* (Mitchill) (Lefevre and Curtis, 1912; Matteson, 1948). *E. complanata* is the most common species of freshwater bivalve encountered in the Atlantic drainage (Clarke and Berg, 1959) and is typically found in lakes, ponds, rivers, and small streams on all types of substrata except very soft mud. Clarke and Berg (1959) reported that often this is the only species found in a particular locality and, if other species of unionids are present, it is the most abundant. Our survey confirms this finding.

Elliptio dilatata (Rafinesque, 1820)

Published Records. Chesapeake Bay drainage. Nanticoke River System: Concord Pond, Sussex Co., DE (Davis, 1984) Station 23).

Elliptio dilatata is an Interior Basin species that closely resembles *E. complanata* and is common throughout its range (Clarke and Berg, 1959) in large and small rivers in either rapid or slow-flowing reaches, as well as in lakes on rocky, gravel, sand, or mud substrata (Clarke and Berg, 1959). Soft tissue anatomy was described by Ortmann (1911). Reproduction occurs in the spring although Ortmann (1919) reported gravid females were found in Pennsylvania from May through August. Glochidia are retained in the marsupium until August (Ortmann, 1919; Clarke and Berg, 1959). The host fish is unknown.

No specimens of *Elliptio dilatata* were found during the present study but a single published record is reported. Since the voucher specimen for this record was not found in the collections of the Academy of Natural Sciences of Philadelphia, it is believed that the specimens were late iden-

tified as *E. complanata*. An examination of the collection locality failed to reveal the presence of this species. Therefore, no verified specimen of *E. dilatata* has been taken from the Delmarva Peninsula.

Alasmidonta undulata (Say, 1817)

Published Record. Chesapeake Bay Drainage. (Station 15) (Clarke, 1981).

Museum Materials Examined. Stations 15 and 17.

Record from Present Study: Station 17.

Clarke (1981) published a single locality for *Alasmidonta undulata* on the Delmarva Peninsula, and two lots from the Choptank River system are at ANSP. We located a single specimen during our survey.

Ortmann (1919) noted that *Alasmidonta undulata* is gravid from July to the following June and Clarke (1981) reported collecting gravid females between August and October. The host fish is unknown. *A. undulata* is reported to occur in moderately flowing streams, from rivers to creeks, and is most abundant on gravel and sand substrata, being absent from mud (Ortmann, 1919; Clarke and Berg, 1959; Clarke, 1981). The species is also found in lakes on sand and gravel substrata (but growth can be stunted in these habitats) and reaches its maximum size in stream outlets located downstream of lakes (Clarke and Berg, 1959). The species is reported to be commonly associated with *Elliptio complanata* and secondarily with *Strophitus undulatus* (Clarke and Berg, 1959; Clarke, 1981). Ortmann (1911) described the soft tissue anatomy.

Alasmidonta varicosa (Lamarck, 1819)

Published Record. Station 47 (Clarke, 1981).

Museum Materials Examined. Station 47.

Clarke (1981) published a single locality for *Alasmidonta varicosa* on the Delmarva Peninsula. However, we were unable to locate the species and a survey of the Clarke's locality failed to reveal the presence of the species.

Alasmidonta heterodon (Lea, 1830)

Museum Materials Examined. Station 17. One lot (ANSP 174899) collected by G. A. Coventry, August 1939, "Delaware."

Although *Alasmidonta heterodon* has been reported from streams to areas adjacent to the Delmarva Peninsula (Clarke and Berg, 1959; Johnson, 1970; Clarke, 1981), it has never been described from the waters of the peninsula-proper. The species was first collected on the peninsula in August 1939 by G. A. Coventry without specific collection data other than "Delaware." One population was found during this study in (Station 17) Norwich Creek, a tributary of Tuckahoe Creek, Choptank River system, near Hillsboro, Maryland. The population is located just within the Talbot Co. line and ap-

pears to be locally abundant. A second population, which we were unable to locate, was reported from Long Marsh Ditch (pers. comm., Maryland Nature Conservancy, 1991). Because of its rare and endangered status, no collections were made.

Alasmidonta heterodon has been described as inconspicuous with a disjunctive distribution along the Atlantic coast (Clarke and Berg, 1959; Clarke, 1981). The species is bradyctic and Clarke and Berg (1959) noted that its breeding season is not well known with gravid females being reported in February and April. Clarke (1981) noted gravid females have been found in June and, in the Tar River, North Carolina, in late August. The fish host species is not known. Details of soft tissue anatomy are presented in Clarke (1981). The reported habitats include medium-sized rivers or rather slow-moving rivers of varying size on substrata of gravel, sand, or muddy sand, and sometimes among submerged aquatic vegetation (Clarke and Berg, 1959; Johnson, 1970; Clarke, 1981). The population at Norwich Creek (Station 17) is living in slow-moving water over a sandy substratum. Clarke and Berg (1959) also noted that *A. heterodon* was associated commonly with *Elliptio complanata* and *Strophitus undulatus* in central New York. Although we found neither of these species in direct association with *A. heterodon* at Norwich Creek, contemporaneous historical collections from this locality indicated the presence of *E. complanata*, *S. undulatus*, as well as *Anodonta cataracta*, *E. fisheriana*, and *A. undulata*.

Anodonta cataracta Say, 1817

Published Record. Seaford, Sussex Co., DE (Rhoads, 1904). Museum Materials Examined. Stations 8, 11, 15, 17, 24, 55, 61. Other materials examined: Leonards Brick Pond, Choptank River System, Cambridge, Dorchester Co., MD. (collections made before 1930, a note with the lot states that the locality now has dwellings) (DMNH 87400): Tributary of Brandywine Creek, Christina River System, Greenville, New Castle Co., DE (ANSP 182963).

One lot from Cambridge, Dorchester Co., MD. (ANSP 132477) is from a fish pond, with no outlets to streams. Another lot (ANSP 355544) gives a locality of "Wye Mills, Norwich Creek, Talbot Co., MD." Wye Mills is located in Queen Anne's Co. and Norwich Creek is in Talbot Co. approximately 8 km east of Wye Mills.

Records from Present Study. Stations 1 - 3, 6, 11, 20, 24, 29, 30, 32, 34, 35, 38, 40 - 42, 51, 52, 55 - 63, 65.

This was the second most commonly found freshwater bivalve on the Delmarva Peninsula. Clarke and Berg (1959) report the species to be common in lakes and ponds and streams varying in size from large rivers to small creeks, it is most abundant on substrata of sand or mud. They further noted that it was the only species found in soft and substrata of ponds and backwater areas. On Delmarva, the species has

been collected historically from small streams but our collections were entirely from the small mill ponds, usually from sand-silt substrata.

Anodonta cataracta is reported to breed from the middle of July to the following April or May (Clarke and Berg, 1959). The host fish species is unknown. Details of soft tissue anatomy are presented in Reardon (1929).

Anodonta implicata Say, 1829

Museum Materials Examined. Stations 4, 5, 11, 13.

One lot (ANSP 355543) gives a locality of "Wye Mills, Norwich Creek, Talbot Co., MD." Wye Mills is located in Queen Anne's Co. and Norwich Creek is in Talbot Co. approximately 8 km east of Wye Mills.

Records from Present Study. Stations 12, 13, 16, 31, 49, 50, 60.

Anodonta implicata is found most commonly in sand or gravel substrata and, very rarely, in mud (Clarke and Berg, 1959; Johnson, 1970). Johnson (1970) notes that the species seems to prefer stream habitat although it can be found in coastal ponds with an unobstructed outlet to the ocean. *A. implicata* was found during our study in ponds without such direct access to the ocean in the Delaware River and Bay drainage (Stations 49, 50, 60).

Ortmann (1919) reported *Anodonta implicata* to be bradytic (winter breeders) with larvae present in the marsupium between July and September. Johnson (1970) reported gravid females in Massachusetts in early May and June. Larvae are released the following spring and the host species is the anadromous alewife, *Alosa pseudoharengus* (Wilson, 1811) (Clarke and Berg, 1959). No report of soft tissue anatomy is known. The glochidia were described by Johnson (1946). Unionids most commonly associated with *A. implicata* are *A. cataracta*, *Lampsilis radiata radiata*, *L. ochracea*, and *Elliptio complanata* (Clarke and Berg, 1959). There are no published records for *A. implicata* on the Delmarva Peninsula.

Lampsilis radiata radiata (Gmelin, 1791)

Published Record. Sussex Co., DE (Davis and Fuller, 1981).

Museum Materials Examined. Stations 3, 5, 11 - 13, 24. Additional materials were examined from Grays Branch, Choptank River System, near Denton, Caroline Co., MD (ANSP 106007).

Collections from Present Study. Stations 1, 2, 12, 13, 33, 44.

Lampsilis r. radiata is distributed widely over the Atlantic Slope (Johnson, 1970). Clarke and Berg (1959) noted that the breeding season appears to begin in August and end the following August. It is not known if this implies continuous breeding or if a hiatus occurs between breeding years.

The host fish is unknown but these authors suggested that many of the species serving as hosts for *L. siliquioidea* (Barnes, 1823) also serve as hosts for glochidia of *L. r. radiata* [bluegill, *Pomoxis annularis* Rafinesque; black crappie, *P. nigromaculatus* (LeSueur); largemouth bass, *Micropterus salmoides* (Lacepede); smallmouth bass, *M. dolomieu* (Lacepede); white bass, *Roccus chrysops* (Rafinesque); yellow perch, *Perca flavescens* (Mitchill); eastern sauger, *Stizostedion canadense* (Smith); and yellow pikeperch, *S. vitreum* (Mitchill)].

Lampsilis r. radiata is found typically on gravel or sand substrata and occasionally on mud. It occurs in lakes and rivers of all sizes but can be absent from smaller ponds and creeks (Clarke and Berg, 1959).

Lampsilis cariosa (Say, 1817)

Published Record. Seaford, Sussex Co., DE (Rhoades, 1904).

Museum Materials Examined. Stations 24, 28.

The anatomy of *Lampsilis cariosa* was described by Lea (1838). Ortmann (1911) found it to be similar to that of *L. ventricosa* (Barnes, 1823). The length of the breeding season is unknown but Clarke and Berg (1959) believed the species to be bradytic. The host species is unknown. *L. cariosa* is found in riffles and shoals of large to medium-sized streams in fine to coarse gravel, usually in sand bars (Clarke and Berg, 1959; Johnson, 1970).

There are only three records for *Lampsilis cariosa* from the Delmarva Peninsula, all from the Nanticoke River system. The species was not encountered during our study even though collections were made at the same stations as the historic records. This absence of *L. cariosa* could not be explained on the basis of misidentification.

Leptodea ochracea (Say, 1817)

Published Record. Seaford, Sussex Co., DE (Rhoades, 1904).

Museum Materials Examined. Stations 5, 24, 28, 55.

Collections from Present Study. Stations 1, 2, 60, 61.

Ortmann (1919) reported *Leptodea ochracea* to be restricted to the tidal portions of the Delaware River. Clarke and Berg (1959) noted the species occurs in ponds, canals, and slow-flowing portions of rivers. The soft tissue anatomy was described by Reardon (1929). Very little is known concerning the glochidia, breeding season, or host species (Ortmann, 1919; Johnson, 1947; Clarke and Berg, 1959) although Johnson (1970) reported finding gravid females in early May at Plymouth, Massachusetts, and thought the species was bradytic. Johnson (1970) also believed that the glochidia probably parasitize migratory fish because *L. ochracea* is restricted generally to the lower reaches of streams having direct connections with the Atlantic Slope.

Ligumia nasuta (Say, 1817)

Published Records. Station 15 and Seaford, Nanticoke River System, Sussex Co., DE (Rhoads, 1904).

Museum Materials Examined. Stations 15, 24, 28. Additional materials: Ditch, Choptank River System, near Petersburg, Kent Co., DE, 1939 (ANSP 175862); headwaters of the Choptank River, Medford, Mills, Sussex Co., DE, 1939 (ANSP 174904).

Record from Present Study. Station 45.

While there are historical collections that have placed *Ligumia nasuta* in various streams and ponds of Delmarva, it was encountered at only a single station (45) during our study.

Clarke and Berg (1959) report *Ligumia nasuta* to breed from August to the following June. The host fish species is unknown. The preferred habitat appears to be ponds, lakes, and slack water portions of streams and canals on sand and mud substrata (Clarke and Berg, 1959). The species is usually associated with *Elliptio complanata* and *Lampsilis radiata*.

Strophitus undulatus (Say, 1817)

Published Record. Station 15 (Rhoads, 1904).

Museum Materials Examined. Stations 13, 17, 47.

Record from Present Study: Station 17.

There is some debate as to the life-cycle of *Strophitus undulatus*. Lefevre and Curtis (1912) and Clarke and Berg (1959) report that the species can complete its development in parental marsupia but note that Baker (1928) reported glochidia completed development after attachment to largemouth bass, *Micropterus salmoides* (Lacepede, 1802), and the northern creek chub, *Semotilus atromaculatus* (Mitchill, 1818). Both of these species are found in waters draining into Chesapeake Bay (Lee, 1980; Lee and Platania, 1980; Hocutt *et al.*, 1986).

The habitats reported for *Strophitus undulatus* include small rivers and creeks on substrata of mud, sand, or fine gravel (Clarke and Berg, 1959; Johnson, 1970). *S. undulatus* is reported in association with *Elliptio complanata* and *Alasmidonta undulata* (Clarke and Berg, 1959). Contemporaneous collections at ANSP confirm this association in Andover Creek, Maryland, as do our own collections in Norwich Creek, Talbot Co., Maryland. These collections further indicate associations with *Lampsilis radiata*, *Anodonta cataracta*, *A. implecata*, *E. fisheriana*, and *Alasmidonta heterodon*.

DIVERSITY

Unionid bivalves were found at 56 (18.2%) of the 307 stations examined (Table 1). Of the 13 species of unionids historically reported from waters of the Delmarva Peninsula, 10 are now present (Table 1). The highest number of species

present at a single locality was 5 (Station 17) followed by one station (Station 2) with 4 species, 8 stations with 3 species, 10 stations with 2 species, and 36 stations with a single species present (Table 1). When these data are compared with those for the 21 historically identifiable stations represented in museum collections or published in the literature only 2 stations show no change in species composition. Additionally, 14 stations show a decline in the number of species present, 2 stations show an increase in species diversity, and 3 stations have lost species but gained new ones. Therefore, the trend is towards a decrease in unionid species diversity.

A review of historical collections revealed that *Elliptio complanata* was associated commonly with *Anodonta cataracta* and *E. fisheriana* and *E. fisheriana* with *A. cataracta* (Table 1). Collections from our survey indicate that *E. complanata* is commonly associated with *E. fisheriana* and *E. fisheriana* with *A. cataracta* but that *E. complanata* now is associated closely with *Lampsilis radiata* (Table 1).

The three most commonly encountered unionids on the Delmarva Peninsula, both historically and at present, are *Anodonta cataracta*, *Elliptio complanata* and *E. fisheriana* (Table 2). Neither *E. dilatata*, *Lampsilis cariosa* nor *Alasmidonta varicosa* were found during our survey. Table 2 summarizes species composition changes for all historical and present collections of unionids on the Delmarva Peninsula.

DISCUSSION

The unionid fauna of Delmarva is composed entirely of species associated with the greater Atlantic Slope fauna (Ortmann, 1919; Clarke and Berg, 1959; Johnson, 1970; Clarke, 1981). The origination of this fauna probably occurred in much the same manner as that of the freshwater fishes of the peninsula. In view of the need for a fish host for the bivalves to complete their development, the population of the peninsula by these two groups should have been simultaneous. Hocutt *et al.* (1986) noted that Chesapeake Bay is the drowned channel of the Susquehanna River and that the lower sea levels associated with interglacial periods facilitated the dispersal of several upland fish species to the peninsula. This process of rising and falling sea levels in the bay occurred many times (Flint, 1957) with the lowest level occurring during the Wisconsinian glaciation (Lougee, 1953). Thus, with the free movement of fishes and unionids during these periods, it is not surprising that Delmarva's unionid fauna is like that of the rest of the Atlantic Slope.

Once established, some isolation of unionid populations in the major drainages of the peninsula could have occurred (Sepkowski and Rex, 1974). Further, extinction of local populations can occur and, given the disjunct populations of such species as *Alasmidonta heterodon*, this has undoubtedly occurred. Given the life cycle and physiological

Table 1. Species present at all stations (A, Species historically present but now absent; B, Species historically present and now present; C, New record for the species).

Taxon	Station Number												
	1	2	3	4	5	6	7	8	9	10	11	12	13
<i>Elliptio fisheriana</i>	—	—	—	—	A	—	—	A	—	—	C	—	A
<i>E. complanata</i>	C	—	A	A	—	C	C	—	C	C	—	B	B
<i>E. dilatata</i>	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Alasmidonta undulata</i>	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>A. varicosa</i>	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>A. heterodon</i>	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Anodonta cataracta</i>	C	C	C	—	—	C	—	A	—	—	B	—	—
<i>A. implicata</i>	—	—	—	A	A	—	—	—	—	—	A	C	B
<i>Lampsilis r. radiata</i>	C	C	A	—	A	—	—	—	—	—	A	B	B
<i>L. cariosa</i>	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Leptodea ochracea</i>	C	C	—	—	A	—	—	—	—	—	—	—	—
<i>Ligumia nasuta</i>	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Strophitus undulatus</i>	—	—	—	—	—	—	—	—	—	—	—	—	A
Taxon	Station Number												
	14	15	16	17	18	19	20	21	22	23	24	25	26
<i>Elliptio fisheriana</i>	—	A	—	B	A	—	—	—	—	A	A	—	—
<i>E. complanata</i>	A	—	—	B	B	C	—	B	B	A	A	C	C
<i>E. dilatata</i>	—	—	—	—	—	—	—	—	—	A	—	—	—
<i>Alasmidonta undulata</i>	—	A	—	B	—	—	—	—	—	—	—	—	—
<i>A. varicosa</i>	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>A. heterodon</i>	—	—	—	B	—	—	—	—	—	—	—	—	—
<i>Anodonta cataracta</i>	—	A	—	A	—	—	C	—	—	—	B	—	—
<i>A. implicata</i>	—	—	C	—	—	—	—	—	—	—	—	—	—
<i>Lampsilis r. radiata</i>	—	—	—	—	—	—	—	—	—	—	A	—	—
<i>L. cariosa</i>	—	—	—	—	—	—	—	—	—	—	A	—	—
<i>Leptodea ochracea</i>	—	—	—	—	—	—	—	—	—	—	A	—	—
<i>Ligumia nasuta</i>	—	A	—	—	—	—	—	—	—	—	A	—	—
<i>Strophitus undulatus</i>	—	A	—	B	—	—	—	—	—	—	—	—	—
Taxon	Station Number												
	27	28	29	30	31	32	33	34	35	36	37	38	39
<i>Elliptio fisheriana</i>	—	A	C	C	C	—	—	C	C	C	C	C	C
<i>E. complanata</i>	C	A	—	C	C	C	—	C	—	—	C	—	—
<i>E. dilatata</i>	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Alasmidonta undulata</i>	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>A. varicosa</i>	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>A. heterodon</i>	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Anodonta cataracta</i>	—	—	C	C	—	C	—	C	C	—	—	C	—
<i>A. implicata</i>	—	—	—	—	C	—	—	—	—	—	—	—	—
<i>Lampsilis r. radiata</i>	—	—	—	—	—	—	C	—	—	—	—	—	—
<i>L. cariosa</i>	—	—	—	A	—	—	—	—	—	—	—	—	—
<i>Leptodea ochracea</i>	—	A	—	—	—	—	—	—	—	—	—	—	—
<i>Ligumia nasuta</i>	—	A	—	—	—	—	—	—	—	—	—	—	—
<i>Strophitus undulatus</i>	—	—	—	—	—	—	—	—	—	—	—	—	—
Taxon	Station Number												
	40	41	42	43	44	45	46	47	48	49	50	51	52
<i>Elliptio fisheriana</i>	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>E. complanata</i>	—	—	—	C	—	—	A	—	C	C	—	—	—
<i>E. dilatata</i>	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Alasmidonta undulata</i>	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>A. varicosa</i>	—	—	—	—	—	—	—	A	—	—	—	—	—
<i>A. heterodon</i>	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Anodonta cataracta</i>	C	C	C	—	—	—	C	—	—	—	—	C	C
<i>A. implicata</i>	—	—	—	—	—	—	—	—	—	C	C	—	—
<i>Lampsilis r. radiata</i>	—	—	—	—	C	—	—	—	—	—	—	—	—
<i>L. cariosa</i>	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Leptodea ochracea</i>	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Ligumia nasuta</i>	—	—	—	—	—	—	—	A	—	—	—	—	—
<i>Strophitus undulatus</i>	—	—	—	—	—	—	—	—	—	—	—	—	—

(continued)

Table 1. (Continued)

Taxon	Station Number													
	53	54	55	56	57	58	59	60	61	62	63	64	65	
<i>Elliptio fisheriana</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	
<i>E. complanata</i>	C	C	—	—	—	—	C	—	C	—	C	—	—	
<i>E. dilatata</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	
<i>Alasmidonta undulata</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	
<i>A. varicosa</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	
<i>A. heterodon</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	
<i>Anodonta cataracta</i>	—	—	B	C	C	C	C	C	B	C	C	A	C	
<i>A. implicata</i>	—	—	—	—	—	—	—	C	—	—	—	—	—	
<i>Lampsilis r. radiata</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	
<i>L. cariosa</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	
<i>Leptodea ochracea</i>	—	—	A	—	—	—	—	C	C	—	—	—	—	
<i>Ligumia nasuta</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	
<i>Strophitus undulatus</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	

requirements of unionids, opportunities for expansion of zoogeographic ranges are limited in these habitats and thus, the observed pattern of distribution of unionids in coastal rivers is not explained easily.

Sepkowski and Rex (1974) offered three hypotheses to explain the distribution of unionid fauna along coastal rivers. The first proposes that gravid mussels could attach to the feet of aquatic birds and are thence transported into neighboring systems. They noted that van der Schalie (1945) rejected this particular statement of the hypothesis but noted that predatory birds can carry the tissues of glochidial-parasitized fish from one stream to another. This form of the hypothesis seems more reasonable given the size and weight of unionid bivalves although the size, depth, and degree of canopy cover of most Delmarva Peninsula streams do not lend themselves to large-scale use by predatory birds.

The second hypothesis hinges on parasitism of secondary and peripheral fishes. Myers (1938, 1951) classified fishes on the basis of their salinity tolerance. Primary fishes have little or no tolerance to sea water; secondary fishes are restricted usually to freshwater but have a salinity tolerance sufficient to cross narrow bands of salt water. Thus, secondary fish hosts could move freely into the bay from one drainage and enter another. Salinity tolerance studies by Musick (1972) and Lee (1976) suggested that primary fishes can tolerate greater salinity concentrations than suggested by Myers. Because of this others (e.g. Hocutt *et al.*, 1986) have suggested that the salinity tolerance classification scheme is invalid. The presumed primary fish host of *Lampsilis r. radiata* and *Strophitus undulatus*, *Micropterus salmoides*, tolerated a salinity of 12.9 ppt and another primary fish host of *L. r. radiata* and *Elliptio complanata*, *Perca flavescens*, tolerated a salinity of 13 ppt. Thus, these fishes could move more freely among the drainage systems of the peninsula than was suspected previously. Unionid glochidia, however, are intolerant of saline conditions (Cvancara, 1970) and their movement on a fish host into saline conditions seems unlikely unless physiological isolation occurs within the fish host's

tissues. It seems more likely that the usual mode of movement of unionid species on fish hosts occurred during times of high freshwater input into the system (e.g. rains, etc.). These events would dilute the saltwater barriers between drainages and this seems to agree with the third hypothesis of Sepkowski and Rex (1974) that stream capture and flooding could play a role in dispersal.

This dispersal mechanism could also explain the absence of unionids on the Virginia portion of the Delmarva Peninsula. These streams are short and shallow and most are tidal and brackish. Furthermore, the waters of the Chesapeake Bay on the west side of the peninsula are more saline [22 - 28 ppt (Bashore and Kelly, 1987)] than those observed farther north in Maryland. The extensive use of mill dams that act to sharply demarcate fresh and salt waters are essentially absent from the Virginia portion of the peninsula. Thus, the movement of unionids from one drainage to another in the southern end of the peninsula, even while attached to a host fish species, requires traversing waters of even higher salinities than those farther north. Even major weather events that result in significant amounts of rainfall could not dilute appreciably waters of these salinities to permit the passage of species from one drainage to another. Another factor contributing to the absence of unionid species in this region is the frequency with which the streams dry up during periods of drought. Should a unionid species become established in a southern peninsula stream, it could find itself without water during the summer months. The absence of species usually associated with ponds (*Elliptio complanata*, *Anodonta cataracta*, *Lampsilis r. radiata*, *Leptodea ochracea*, *Ligumia nasuta*) is puzzling because many of the existing ponds have been stocked with fish.

The collections made during our survey indicate a general trend toward decline in species diversity. While there has not been a historical trend towards development of heavy industry on the Delmarva Peninsula during the past 100 years, there has been an increase in population and an intensification of agricultural production. This has led to the channel-

ization of many streams for both flood control and increased drainage of farm fields. This process could have contributed to habitat loss for some species because several historical localities are now channelized. Furthermore, the increased application of agricultural chemicals could have played a role in diversity decline by acting either directly upon the mussels or upon their fish hosts species. Because many of the fish hosts for the species of unionids on Delmarva are as yet unknown, the extent of this factor is unknown.

The zebra mussel, *Dreissena polymorpha*, has yet to be found along the Delmarva Peninsula. The point at which direct introduction from abroad is likely to occur is the Chesapeake Bay and Delaware Canal. The Maryland Department of Natural Resources is currently monitoring these waters for evidence of this exotic species.

ACKNOWLEDGMENTS

The authors wish to thank Gene Handwerker and Nicholas Counts for their help during sampling. Thanks are also due David O'Neill, University of Maryland Eastern Shore, for his help in collecting specimens. We would like to also thank Dr. Rüdiger Bieler, Delaware Museum of Natural History and Dr. Arthur E. Bogan, Academy of Natural Sciences of Philadelphia, and their respective staffs for assisting us in locating historical materials described in this paper. The paper benefited from the comments of two anonymous reviewers whose assistance is gratefully acknowledged.

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Date of manuscript acceptance: 17 May 1991

APPENDIX.

Collection station numbers and localities examined for the presence of unionid bivalves on the Delmarva Peninsula. The geographic locations of stations are identified in figure 1 by station number. Stations are listed from north to south first in the Chesapeake Bay drainage and secondly in the Delaware Bay Atlantic Ocean drainage. Previously reported stations are referenced in parentheses. (DMNH, Delaware Museum of Natural History; ANSP, Academy of Natural Sciences of Philadelphia; UMES, University of Maryland Eastern Shore). Only those stations where unionid bivalves were found are listed. A complete list of all station localities, including those where no naiades were found, is on file at ANSP.

Chesapeake Bay Drainage

SUSQUEHANNA RIVER

1. Susquehanna River, mud flat at Veterans Hospital, Perry Point, Perryville, Cecil Co., MD (UMES 2258, 2259, 2260, 2261)

BOHEMIA RIVER SYSTEM

2. Little Bohemia Creek, Bohemia Mills, Bunker Hill Road, Cecil Co., MD (UMES 2397, 2398, 2399)

SASSAFRAS RIVER SYSTEM

3. Sassafras River, MDSR 299 bridge, Cecil-Kent Co. line, MD (ANSP 358302, 358303, 358304, 358305; UMES 2408)
4. Sassafras River, under US 301 bridge, Cecil-Kent Co. line, MD (Davis, 1984; ANSP 346882, 346889, 347868)

CHESTER RIVER SYSTEM

5. Ratcliff Creek, MDRT 661, Chestertown, Kent Co., MD (Ortmann, 1919; ANSP 65093, 65095, 65096; DMNH 78556)
6. Island Creek, MDSR 213 bridge, north of Centreville Queen Anne's Co., MD (UMES 2402, 2403)
7. Granny Finley Branch, MDSR 213 bridge, Queen Anne's Co., MD (UMES 2409)
8. Southeast Creek, MDSR 213 crossing, Queen Anne's Co., MD (Ortmann, 1919; ANSP 66311)
9. Red Lion Branch, Dudley Corse Road, Queen Anne's Co., MD (UMES 2395)
10. Red Lion Branch, Red Lion Branch Road, Queen Anne's Co., MD
11. Unicorn Branch, Unicorn Community Lake, Queen Anne's Co., MD (ANSP 358299, 358300, 358301; UMES 2378, 2379)
12. Chester River, MDSR 313 bridge, Millington, Kent-Queen Anne's Cos., MD (ANSP A9484, 358297, 358298, 358308; UMES 2307, 2308, 2309)
13. Andover Branch, Peacock Corner Road crossing, east of Millington, Kent Co., MD (ANSP A9482, 355555, 355801, 358291, 358292, 358293; UMES 2480, 2481, 2482)
14. Chester River, US 301 bridge, Kent-Queen Anne's Co. line (ANSP 346890)

CHOPTANK RIVER SYSTEM

15. Choptank River, "Choptank Mills," RT 207 at Mud Mill Pond, Kent Co., DE (Rhoads, 1904; Clarke, 1981; ANSP 85224, 85226, 85264)
16. Tuckahoe Lake, Tuckahoe Creek, Tuckahoe State Park, Crouse Mill Road bridge, Caroline-Queen Anne Cos., MD (UMES 2375)
17. Norwich Creek, RT 404 bridge, Queen Anne's Co., MD (ANSP A9487, A9488, A10261, A10262, 355556, 355802, 358285, 358286, 358287, 358288, 358289, 358290; DMNH 41138; UMES 2471, 2472, 2473, 2474)
18. Mason Branch, Tuckahoe Creek, MDSR 304 bridge, Caroline-Queen Anne's Cos., MD (ANSP 358294, 358295, 358296; UMES 2265)
19. Watts Creek, Legion Road bridge, Caroline Co., MD (UMES 2380)
20. Williston Lake at spillway, Denton Road, Caroline Co., MD (UMES 2400)
21. Hog Creek, Hog Creek Road bridge, Caroline Co., MD (ANSP 358306; UMES 2483)
22. Beaverdam Branch, 1.5 km west of Matthews at MDSR 328 bridge, Talbot Co., MD (ANSP 358309; UMES 2484)

NANTICOKE RIVER SYSTEM

23. Concord Pond, just north of DESR 20, CR 516, 1.6 km east of Seaford, Sussex Co., DE (Davis, 1984; ANSP 345054, 352551, 358277)
24. "Deep Creek," just below Concord Pond spillway, RT 516, east of Seaford, Sussex Co., DE (Davis, 1984; ANSP 346863, 346864, 346865, 346866, 346867, 347863, 347864, 347865, 347866, 347867, 349333, 349337, 349338, 358272, 358273, 358274, 358276, 358278; UMES 2476)
25. Gravelley Branch, Collins Pond, just east of Coverdale Crossroads, Sussex Co., DE (UMES 2243)
26. Marshyhope Creek, DESR 16 bridge, just east of Hickman, Kent Co., DE (UMES 2233)
27. Nanticoke Branch, DESR 18 bridge, just east of junction with CR 533, Sussex Co., DE (UMES 2235)
28. Nanticoke River, US 13 bridge, Seaford, Sussex Co., DE (Rhoads, 1904; ANSP 84837, 88219, 88370, 301003, 301004, 345058; DMNH 75214)
29. Trap Pond, Trap Pond State Park, Sussex Co., DE (UMES 2266, 2267)
30. Chipman Pond, CR 465 bridge at dam, Sussex Co., DE (UMES 2262, 2263, 2264)
31. Meadow Branch, just downstream of Horsey's Pond, DESR 24 bridge, Laurel, Sussex Co., DE (UMES 2276, 2277, 2279)
32. Quantico Creek, Quantico Creek Road, east of Quantico, Wicomico Co., MD (UMES 2469, 2470)
33. Rewastico Pond, Rewastico Creek at dam, Athol Road, Wicomico Co., MD (UMES 2252)
34. Barren Pond, Barren Creek at dam, Wicomico Co., MD (UMES 2249, 2250, 2451)
35. Tyndall Branch, Fleetwood Pond, CR 484 at dam, Sussex Co., DE (UMES 2298, 2297)
36. Record Pond, at dam, Laurel, Sussex Co., DE (UMES 2293)
37. Gales Creek, Galestown Pond, Galestown-Reliance Road at spillway, Galestown, Dorchester Co., MD (UMES 2287, 2289)

38. Butler Mill Branch, Craigs Pond, public boat access at spillway, CR 542A, Sussex Co., DE (UMES 2290, 2291)
39. Nanticoke River, Williams Pond, Tharp Road bridge, Seaford, Sussex Co., DE (UMES 2300)
40. Bucks Branch, Hearn Pond, CR 544A at spillway, Sussex Co., DE (UMES 2299)
41. Smithville Lake, Opossum Hill Road at dam, Caroline Co., MD (UMES 2377)

WICOMICO RIVER SYSTEM

42. Wicomico River, Riverside Boat Ramp Park, Salisbury, Wicomico Co., MD (UMES 2241)
43. Leonard Pond Run, Leonard Pond, Leonard Pond Park, US 13 bridge, Salisbury, Wicomico Co., MD (DMNH 48638)

POCOMOKE RIVER SYSTEM

44. Nassawango Creek, Red House Road bridge, Worcester Co., MD (UMES 2374)
45. Unnamed tributary (A) of Nassawango Creek, Nassawango Road just southeast of Pennewell Road, Worcester Co., MD (UMES 2219)

Delaware River and Bay, Atlantic Ocean Drainage**CHRISTINA RIVER SYSTEM**

46. Brandywine Creek, Brandywine Park at Dam near Brandywine Zoo, Wilmington, New Castle Co., DE (DMNH 44090)
47. Red Clay Creek at Yorklyn, New Castle Co., DE (Clarke, 1981; ANSP 85227, 85228)
48. Becks Pond, SR 48 at dam and swimming beaches, New Castle Co., DE (UMES 2269, 2270)

APPOQUINIMINIK RIVER

49. Shallcross Lake at Greylag, CR 428 bridge at dam, New Castle Co., DE (UMES 2282, 2283)
50. Silver Lake, CR 442 bridge at dam, east of Middletown, New Castle Co., DE (UMES 2281)
51. Wiggins Mill Pond, Road 446, below spillway, New Castle Co., DE (UMES 2406)

BLACKBIRD CREEK

52. Pond, Road 463A, below spillway, New Castle Co., DE

SMYRNA RIVER SYSTEM

53. Paw Paw Branch, Road 40 bridge, east of Thomas Corner, New Castle Co., DE (UMES 2396)

LEIPSIC RIVER SYSTEM

54. Massey's Millpond, Road 42 bridge, Kent Co., DE (UMES 2407)

ST. JONES RIVER SYSTEM

55. Silver Lake, Silver Lake Recreation Area, Dover, Kent Co., DE (ANSP 45991, 45992; UMES 2376)
56. Tidbury Creek, Derby Pond, US 13A, south of Wyoming, Kent Co., DE

MURDERKILL RIVER SYSTEM

57. McGinnis Pond, Road 378, southwest of Lexington Mill, Kent Co., DE (UMES 2393)
58. McColley Pond, DESR 15 bridge at dam and spillway, Mordington, Kent Co., DE (UMES 2392)

MISPILLION RIVER SYSTEM

59. Blairs Pond, Road 443, west of Milford, Kent/Sussex Cos., DE (UMES 2382, 2385)
60. Griffith Lake, Road 443 at spillway, west of Milford, Kent/Sussex Cos., DE (UMES 2384, 2386, 2388)
61. Haven Lake, US 113 bridge at spillway, Milford, Kent/Sussex Cos., DE (ANSP 103011; UMES 2389, 2390, 2391)
62. Silver Lake, DESR 36 bridge at spillway, Milford, Kent/Essex Cos., DE (UMES 2401)

CEDAR CREEK SYSTEM

63. Cedar Creek, Cabbage Pond, CR 214 bridge, Sussex Co., DE (UMES 2284, 2285)

INDIAN RIVER SYSTEM

64. Millsboro Pond, DESR 24 at dam, Millsboro, Sussex Co., DE (ANSP 85835)
65. Ingram Pond, Public Fishing Area at dam, CR 328, Sussex Co., DE (UMES 2298)

The influence of oxygen availability on oxygen consumption in the freshwater clam *Musculium partumeium* (Say) (Bivalvia: Sphaeriidae)

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Abstract. The O₂ consumption of the freshwater clam *Musculium partumeium* Say was determined as a function of clam size and O₂ availability. The rate of O₂ consumption by large individuals was influenced significantly by O₂ availability whereas the rates of smaller individuals were not altered significantly. One reason for the difference in response for large and small animals was the greater degree of availability in O₂ consumption among smaller individuals.

A number of studies have examined the influence of low O₂ availability on metabolism in marine bivalves (e.g. Newell, 1979 and references therein). Much of this work is predicated upon the supposition that the tidal exposure of many bivalves in marine habitats regularly subjects them to periods of low O₂ availability and, thus, these forms could have responded evolutionarily to this selection pressure.

Few studies, however, have examined the influence of reduced O₂ availability in freshwater bivalves (Burky, 1983; Burky *et al.*, 1985; Hornbach, 1985). While there is not a cyclical tidal change in O₂ availability in freshwater systems, there are a number of factors which could affect O₂ accessibility. Diurnal rhythms of O₂ concentration in littoral areas are known to occur as a result of shifts in the dominance of photosynthetic or respiratory activities in these highly productive areas. Seasonal shifts in O₂ availability in the profundal zones of lakes associated with temperature stratification and subsequent turnover events are also well documented. Probably one of the most severe of freshwater habitats, in terms of changes in O₂ availability, are ephemeral ponds. In these habitats, O₂ levels can be high in the spring as the pond fills, but as water levels decline in the mid to late summer, O₂ availability often becomes restricted. Also, many ephemeral ponds have heavy loads of organic debris which can contribute to anoxic conditions in very shallow water during any season.

One of the most common inhabitants of ephemeral ponds are bivalves of the family Sphaeriidae, the fingernail and pea clams. Within this family, the genus *Musculium* has the largest population of species found in temporary habitats (Burch, 1975). A moderately large body of information is available concerning life history adaptations in this genus for survival in ephemeral ponds (see e.g. Hornbach and

Childers, 1986), yet little information is available concerning physiological adaptations for this harsh habitat (Burky, 1983). This study examines the influence of O₂ availability and body size on O₂ consumption in the clam, *Musculium partumeium* (Say).

MATERIALS AND METHODS

Clams utilized in this study were collected in May - July 1986 from a temporary pond located at the Kathryn Ordway Natural History Area in Inver Grove Heights, Minnesota. In all cases, clams were removed from the pond when sediment temperatures were near 17°C (range 16.5 - 18.0°C). Specimens were returned to the laboratory and maintained at 17°C in filtered pond water (Whatman "42", effective pore size 2.5 µm) for 6-12 hr before metabolic rates were determined. The shell length (anterior-posterior dimension) was measured to the nearest 0.1 mm with a dissecting microscope fitted with a stage mounted micrometer.

To examine the effect of size and O₂ availability on the metabolic rate of *Musculium partumeium*, the following experimental design was utilized. The metabolic rates for three replicates of each of four size groups of clams were determined at six different levels of O₂ availability. This yielded 72 values of O₂ consumption.

Water with various levels of O₂ availability were prepared by mixing air-saturated, filtered pond water with nitrogen-saturated, filtered pond water in proportions of 1:0, 3:1, 1:1, 1:3, 1:7 and 0:1, thus giving six levels of O₂ availability. Because of the variability in the mixing process, changes in barometric pressure, etc., mixtures prepared on different days had varying Po₂s. The averages and ranges (in parentheses) of Po₂ values [measured by the azide-modified

Table 1. Summary of numbers, sizes and range of metabolic rates of *Musculium partumeium* utilized in this study. The coefficient of variation was calculated as the standard deviation as a percentage of the mean.

Experiment Number	Number of clams per bottle	Mean shell length	Mean ash-free dry weight (mg)	Range of metabolic rates ($\mu\text{l O}_2 \text{ AFDW}^{-1} \text{ hr}^{-1}$)	Mean coefficient of variation (%)
1	10	1.2	0.05	0.68-4.78	35.7
2	5	2.6	0.31	0.44-2.26	33.3
3	5	4.0	0.79	0.81-1.46	8.3
4	3	6.2	2.59	0.62-1.41	18.5

Winkler method (American Public Health Association, 1980)] were 140 mm Hg (117-166), 116 mm Hg (98-129), 93 mm Hg (75-109), 71 mm Hg (57-85), 58 mm Hg (42-72) and 41 mm Hg (27-50) for the mixtures (air-saturated water: nitrogen-saturated water) 1:0, 3:1, 1:1, 1:3, 1:7 and 0:1, respectively.

O₂ consumption of groups of 3-10 clams (depending on size) (Table 1) was measured after the specimens had been placed in 40 ml glass-stoppered bottles containing one of the pond water mixtures at 17°C. Control chambers (bottles with no clams) were analysed similarly as controls to correct for changes in O₂ concentration not directly attributable to clam metabolism. After 15-20 hr, the O₂ remaining in each chamber was measured with the azide-modified Winkler method. Duplicate 10.0 ml aliquots of water from each chamber were titrated with approximately 0.0025 N sodium thiosulfate (standardized with potassium bi-iodate) and a digital buret calibrated to 0.01 ml. Results were corrected for the volume of Winkler reagents used (0.5 ml each) in the fixation process. Additional details of this method can be found in Hornbach *et al.* (1983). Values as mg O₂ were converted to $\mu\text{l O}_2$ or Po₂ (mmHg) at STP with equations presented in Pierce *et al.* (1973). Ash free dry weights (AFDW) were determined for all groups of clams as the difference in weight after drying to constant weight at 100°C and ashing at 500°C.

RESULTS

Figure 1 shows the effect of O₂ availability on the rate of O₂ consumption for each of the four size categories of *Musculium partumeium* utilized in this study. Multiple regression analysis for each size class, with $\log_e(QO_2)$ ($\mu\text{l O}_2 \text{ mg AFDW}^{-1} \text{ hr}^{-1}$) as the dependent variable and Po₂ (O₂ tension mm Hg) and $\log_e(\text{AFDW})$ as the independent variables, showed that Po₂ significantly influenced QO₂ for the two largest size classes (4.0 mm - $F=17.0$, 1,16 df, $p=0.0009$; 6.2 mm - $F=4.77$, 1,17 df, $p=0.045$). For the two smaller size classes, there was no significant influence of Po₂ on QO₂ (1.2 mm - $F=1.44$, 1,17 df, $p=0.248$; 2.6 mm - $F=1.35$, 1,17 df, $p=0.263$). This lack of a statistically significant influence was due partially to the large degree of variability

in the O₂ consumption rates for these size groups of clams (Table 1). An overall regression analysis resulted in the following equation: $\log_e(QO_2) = -0.21 - 0.169 [\log_e(\text{AFDW})] + 0.003 (\text{Po}_2)$; $r^2=0.44$ ($F=26.37$, 2,70 df, $p=0.0001$). The standardized partial regression coefficients for $\log_e(\text{AFDW})$ and Po₂ are -0.66 and 0.28, indicating that AFDW had a higher degree of influence on metabolic rate than did O₂ availability.

Bayne (1971) derived an index for assessing the degree of influence of O₂ availability on O₂ consumption. If the consumption availability curve is hyperbolic, then $QO_2 = \text{Po}_2 / (k_1 + k_2 (\text{Po}_2))$. When Po_2 / QO_2 is plotted against Po₂, a straight line is produced with k_1 the intercept and k_2 the slope. A high value of the ratio k_1/k_2 indicates a greater O₂-dependence of O₂ consumption. Regressions of these values were performed for each size class of clam to obtain estimates of k_1 and k_2 . The results are shown in Table 2.

DISCUSSION

Several studies have shown that sphaeriid clams are capable of surviving various periods of anoxia (Juday, 1908;

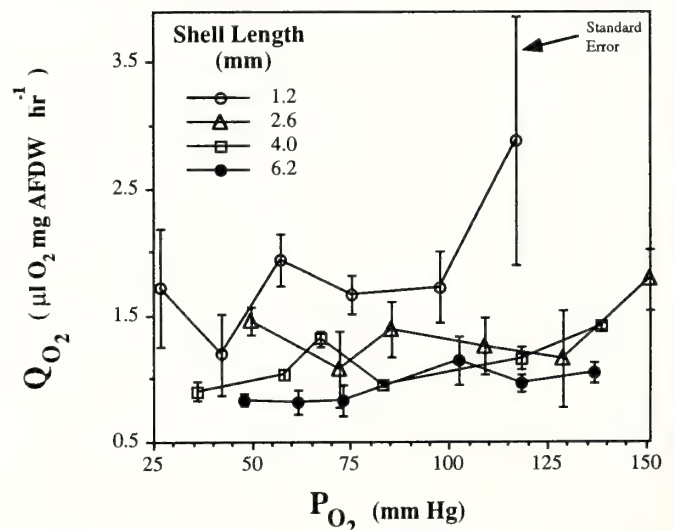


Fig. 1. Relationship between oxygen availability and oxygen consumption for four sizes of *Musculium partumeium*.

Table 2. Indices of oxygen dependence of metabolism for various sizes of *Musculium partumeium*. Indices are from the equation $Po_2/Qo_2 = k_1 + k_2$ (Po_2) and r^2 is the coefficient of determination.

Mean Shell Length (mm)	k_1	k_2	r^2	k_1/k_2
1.2	14.95	0.38	0.36	39.45
2.6	17.77	0.68	0.21	26.21
4.0	19.54	0.64	0.80	30.63
6.2	26.83	0.78	0.66	34.53

Eggleton, 1931; Thomas 1963, 1965; Gale, 1976; Holopainen and Jónasson, 1983; Way *et al.*, 1983; Holopainen, 1987). Nonetheless, few studies have examined the influence of O_2 availability on metabolism (Hornbach, 1985). Waite and Neufeld (1977) indicated that for the sphaeriid, *Sphaerium simile* (Say), there was little influence of O_2 availability on O_2 consumption over the range of 32-80 mm Hg Po_2 . Outside this range the influence of O_2 availability on O_2 consumption varied with temperature. Berg *et al.* (1962) found that O_2 consumption declined and reduced O_2 availability in *Pisidium casertanum* (Poli). Buchwalder (1983) found that *Musculium partumeium* and *M. lacustre* (Müller) displayed a dependence of O_2 consumption on O_2 availability and that this dependence varied seasonally. The greatest independence was found during periods of low O_2 availability and could help to explain how some sphaeriids can grow and reproduce under hypoxic conditions (Thomas, 1963, 1965; Way *et al.*, 1980). In this study I found that there is an effect of O_2 availability on O_2 consumption and that the effect appears to be size dependent (Fig. 1). There was considerable variability in the rates of consumption that were ascertained (Table 1). This high degree of variation could be due to behavioral differences in individuals (e.g. differences in locomotory or pumping activities) or differences in the reproductive condition of mature individuals. No attempt was made to observe individual variation in these parameters, however it was noted that in the vast majority of cases, all individuals were moving actively in the chambers, though it is unknown if this occurred continuously throughout the 15-20 hr experimental period. The fact that the variation in O_2 consumption is not significantly higher for brooding clams (6.2 mm size class - Table 1) suggests that reproductive condition does not alter significantly the variation noted by this technique.

Newell (1979), based on studies of marine bivalves by Bayne (1971, 1973) and Taylor and Brand (1975), claimed that small animals, with high weight-specific metabolic rates tend to have higher k_1/k_2 ratios than larger conspecific animals, indicating a decreased ability in smaller organisms to regulate O_2 consumption. Based on the k_1/k_2 values in Table 2, *Musculium partumeium* ≥ 2.6 mm appears to follow an opposite trend, that is, larger animals tend to have larger values

of k_1/k_2 indicating a greater independence of O_2 consumption on O_2 availability. *M. partumeium* in the 4.0 and 6.2 mm size classes are reproductively active and often contain extra-marsupial larvae (Hornbach *et al.*, 1980; unpubl data). It is possible that the larvae displayed a regulatory pattern from the adults and thus mask the response of the adult. For example, Burky and Burky (1976) have shown that over 50% of the O_2 consumption of adults of the sphaeriid, *Pisidium walkeri* Sterki, could be attributed to the respiration of incubating embryos.

Although the 1.2 mm clams had the highest k_1/k_2 ratio (Table 2), the r^2 of the relationship used to attain this value was low (Table 2). In fact, for clams of mean shell length 1.2 or 2.6 mm, there was no statistically significant influence of O_2 availability on O_2 consumption. This could indicate that these size clams are regulators. However, Table 1 indicates that one reason for the lack of a statistical relationship between O_2 availability and O_2 consumption in these size groups is due to the high individual variation in metabolic rates. The reasons for this variability are not known. However, in temporary ponds, many juvenile individuals do not grow during the summer. Apparently this lack of growth is required because only juveniles can overwinter in the dry pond (Way *et al.*, 1980; Hornbach and Childers, 1986). These overwintering juveniles grow to adults in the spring, reproduce and die during the summer. There is some evidence, however, that if temporary ponds remain wet through the fall, some portion of the juveniles produced in the spring and summer grow and reproduce in the fall (Hornbach *et al.*, 1980). There are varying genotypes in the population (McLeod *et al.*, 1981). It is possible that each is associated with a different metabolic rate as juvenile and this contributes to the observed variability (Table 1). The fact that juvenile *Musculium partumeium* must survive extremely long periods of anoxia, compared to many marine bivalves, could explain why their patterns of O_2 consumption dependency on O_2 availability do not follow those described by Newell (1979) for marine species.

The results of this study indicate that O_2 availability can influence O_2 consumption in *Musculium partumeium* but that the influence is size-specific. Furthermore it appears that the degree of variability in O_2 consumption is also influenced by size.

ACKNOWLEDGMENTS

I wish to thank Karen Moulton, Tony Deneka and Robert Dado for their assistance in collecting specimens and technical assistance in the laboratory.

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Date of manuscript acceptance: 9 July 1991

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4 - 5 JUNE 1990

Sexual conflict and the mating systems of simultaneously hermaphroditic gastropods

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Abstract. Here I review the predictions, for hermaphroditic gastropods, of recent developments in mating systems and sexual conflict theory. Sexual conflict theory predicts that hermaphrodites should have a species-specific preferred sexual role. The Hermaphrodite's Dilemma model explores the consequences of this and predicts that all hermaphrodite mating systems should be based on reciprocity with cheating in a preferred role. Traditional models based on Bateman's principle predict that the male role will be preferred. Experimental and observational evidence from *Navanax inermis* (Cooper) indicate that the female role is preferred, contrary to predictions from Bateman's principle, and that the mating system is based on sperm-trading which serves to enforce reciprocity, preventing individuals from specializing in the female sexual role. Comparison of *Navanax* to other hermaphrodites suggests that the preferred sexual role is that which offers control of fertilization. This, the gamete-trading model, predicts that all hermaphroditic gastropods with sperm storage and a gametolytic gland should demonstrate a preference for the female role and a mating system based on sperm trading. This model and the Hermaphrodite's Dilemma model of strategies in a situation of sexual conflict make specific predictions about the behavior of hermaphroditic gastropods. The available literature on opisthobranchs and pulmonates suggests several interesting tests of these models but the available data are insufficient to support or refute the predictions. The mating systems of euthyneuran gastropods require investigation from the standpoint of modern mating systems theory.

Recent progress in understanding the evolution of species-typical reproductive behaviors stems largely from the analysis of mating systems in terms of a conflict of interests between the sexes (Orians, 1969; Trivers, 1972; Emlen and Oring, 1977; Parker, 1979; Hammerstein and Parker, 1987). Because such sexual conflict is assumed to be a product of the differential selective pressures associated with reproduction through sperm versus reproduction through eggs (Parker, 1979), it should exist in all anisogamous organisms (Bateman, 1948). Although work in this field (despite Ghiselin, 1974; Williams, 1975) has concentrated on a small number of taxa (largely vertebrates and insects), almost all of which have separate sexes, there has been increasing interest in the role of sexual conflict and/or sexual selection in the mating systems of a wider array of organisms, such as plants, including hermaphroditic forms (Willson and Burley, 1983; Bronstein, 1988; Galen and Rotenberry, 1988; Nakamura and Stanton, 1989) and some animals with sequential hermaphroditism (primarily fish, see Charnov, 1982, 1986; Shapiro and Boulon, 1982; Warner, 1982; Warner and Lejeune, 1985) and more recently simultaneous hermaphroditism (e.g. serranid fishes; Fischer, 1980, 1984; Fischer and Petersen, 1987; and the polychaete, *Ophiotrocha*; Berglund, 1985; Sella, 1985, 1988).

Extension of the analysis of sexual conflict to a diverse array of taxa and modes of sexuality is important for two

reasons: 1) it could provide new and useful insights into the biology and evolutionary ecology of taxa of interest; 2) by examining the mating systems of a diverse array of organisms in terms of sexual conflict and/or sexual selection, we should be able to identify useful systems for testing some of the assumptions and predictions of modern mating systems theory.

The gastropods are particularly interesting in this respect because they offer: 1) a diverse array of sexual systems and reproductive strategies; 2) a variety of very complex reproductive behaviors; 3) complex genitalia, the anatomy of which is important taxonomically in many groups. Where genital anatomy is varied sufficiently to be a useful taxonomic character at the levels of genus, subgenus and species, as in some groups of gastropods (Mead, 1943; Rudman, 1974; Gilbertson, 1989; Patterson, 1989), sexual selection is likely to have been important (Eberhard, 1985). Similarly, where there are elaborate forms of courtship and copulatory behavior, and particularly where there is significant diversity within a taxonomic group, one predicts that sexual conflict and/or sexual selection has been important as a selective force.

In this paper, I 1) present predictions as to the types of sexual behavior and mating systems expected for hermaphroditic gastropods if sexual conflict is important and 2) discuss a few well-known sexual phenomena in the two predominantly hermaphroditic subclasses of gastropods (Opisthobranchia and Pulmonata) in light of predictions of

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recent models based on the assumption of sexual conflict. These two subclasses, collectively (loosely) termed the euthyneuran gastropods, are predominantly outcrossing simultaneous hermaphrodites. Hermaphroditic species offer exciting opportunities to test: 1) the hypothesis that sexual conflict exists (Leonard, 1990), because as Parker (1979) has pointed out, sexual conflict has been widely assumed but there is little evidence that it exists; 2) alternate hypotheses as to the source and nature of sexual conflict (Leonard and Lukowiak, 1991).

SEXUAL CONFLICT

SEXUAL CONFLICT AND HERMAPHRODITE MATING SYSTEMS

Sexual conflict is a conflict of interests between the two parties to a mating encounter such that one individual has more to gain (less to lose) by mating than the other does. The idea of sexual conflict is based on the common observation that among many species of animals, males are "eager" to mate with virtually any available female, to the extent of risking death and/or serious injury in fighting other males for access to females, whereas females are "coy". This phenomenon, which Darwin (1874) considered paradoxical, is usually explained by Bateman's (1948) principle, i.e. that males are more eager to fertilize eggs than females are to get their eggs fertilized because the fitness of females is limited by the resources available for egg production, whereas the fitness of males is only limited by the availability of females.

For a simultaneous hermaphrodite, sexual conflict arises if and when there is more to be gained from mating in one sexual role than in the other. In the population as a whole, reproductive success through eggs must exactly equal reproductive success through sperm (R. A. Fisher, 1958). However, the distribution of reproductive success across the population could well differ for sperm and eggs (i.e. the variances differ, see Charnov, 1979). When this is the case, there is a potential asymmetry in the pay-offs of the two sexual roles to an individual, particularly in a single encounter, and an individual's overall fitness (its reproductive success relative to the rest of the population) will depend in part on how it divides its reproductive effort (or reproductive opportunities) between the two sexual roles. That is, an individual which specializes (differs from the population average of 50:50 reproduction through sperm versus eggs) in one of the roles (that which is less costly in terms of energy expenditure, mating time, risk, etc., see below) could be able to achieve greater than average fitness. This means of course that other individuals in the population will find themselves specializing inadvertently in the more costly role, which should result in below average fitness.

Charnov (1979:2482) discussed the implications of Bateman's principle for pair-mating hermaphroditic animals,

including gastropods, and recognized that, in simultaneous hermaphrodites; "There must often be a conflict of interest between mating partners — as a recipient each should be inclined to accept sperm (not necessarily for fertilization of its own eggs) in order to give its sperm away. As a donor, one should be selected to induce one's partner to use the new sperm in fertilization". He went on to suggest that both the complicated reproductive anatomy and the elaborate precopulatory behaviors of animals such as gastropods "are explicable when one realizes that the interests of the partners are often in conflict". In hermaphrodites this sexual conflict is direct, in that each individual is in direct competition with all other individuals, including its mate, for fitness. On the other hand in dioecious species sexual conflict (with the exception of conflict over parental care) is an epiphenomenon of intrasexual competition (Hammerstein and Parker, 1987).

Thus, in simultaneous hermaphrodites sexual conflict should lead to the evolution of a preference for mating in a particular sexual role, a preference that will be shared by all individuals of the species. If all individuals prefer the same role, the interests of two individuals meeting for a mating encounter will be in direct conflict. In hermaphrodites, each mating encounter could be expected to involve competition between members of the pair for the preferred role. Mating systems in hermaphrodites should reflect or represent a resolution of, this competition for the preferred role (Leonard, 1990, unpub. data; see also below).

THE ORIGIN OF SEXUAL CONFLICT

If sexual conflict exists, the preferred sexual role will be consistent within a species, since the term implies an inherent advantage to one sexual role, but the favored role could vary between species, depending on the source of sexual conflict. Charnov based his arguments (Charnov, 1979, 1982) on the explicit assumption of Bateman's principle, i.e. that male fecundity is limited by access to eggs while female fecundity is not limited by sperm availability. While Bateman's principle is the most widely accepted explanation of sexual conflict, there are two general types of alternative explanation: mating time or rate (Baylis, 1981; Sutherland, 1986); and control of fertilization or risk (Alexander and Borgia, 1979; Leonard and Lukowiak, 1984, 1985, 1991). The first of these, the rate hypothesis, argues that a discrepancy between males and females in the minimum interval between matings, could be a source of sexual conflict. That is, if after a mating encounter, one sex (say the female) must spend a prolonged period in yolking up eggs, or some form of parental care (gestation, brooding, etc.) before being able to increase her fitness by a second mating encounter, whereas the male can inseminate many females in that same time period then one could expect the female to be "coy" and the male, "eager", even in the absence of differential energy expenditure. For a discussion of how mating rate can favor the evolution of

male parental care see Baylis (1981).

The second type of hypothesis is based on the idea that the important dichotomy between reproducing through eggs and reproducing through sperm may not be differential costs in a fixed currency (such as energy or time) but rather a differential probability that the investment that is made will pay off in the form of zygotes. If gamete production involves use of an exhaustible resource, then in economic terminology, optimal depletion of that resource (i.e. depletion which maximizes profit) must be based not only on accounting costs (the energy, time, etc. required for a particular type of reproductive act) but also the opportunity cost (that is the cost of a reproductive act now must include the value of that act if it were made at some future date, or the pay-off that could have been achieved from the other role, etc.) (Nicholson, 1978; A. C. Fisher, 1981). Alexander and Borgia (1979) suggested that an important difference between the sexes could be the extent to which control is exerted over the fate of the gametes that they produce. Alexander and Borgia (1979) argued that females, in general, produce fewer gametes not because they are limited by energy availability but rather because they invest more in "following-up" on that gamete, insuring that it will be fertilized. That is, one can think of females as adopting a "risk-averse" reproductive strategy whereas males have a "risk-prone" reproductive strategy. This type of model has some interesting implications for hermaphrodites.

The unique feature of hermaphrodite sexual strategies is obviously the opportunity to choose between reproduction through eggs or sperm. If, as is required for sexual conflict, the variances of these two types of strategy differ, which should be preferred, the high variance strategy or the low variance strategy? Extension of Bateman's principle to hermaphrodites (Bateman, 1948; Charnov, 1979) suggests that the male role (the high variance strategy) will be preferred (see above). However, Gillespie (1977) has demonstrated that where two genotypes yield equal average offspring number, but have unequal variances, the one with the lower variance will offer a fitness advantage. This suggests that given an opportunity to choose between a high and a low variance reproductive strategy, selection would favor the individual that opted for the low variance strategy (Leonard and Lukowiak, 1991). In Gillespie's (1977: 1012) words, "the addition of a stochastic element to the offspring number of a genotype will effectively lower the fitness of that genotype as measured by its mean frequency in the next generation". A practical problem in using Gillespie's principle to predict the favored sexual role for a hermaphroditic species is that we have little empirical evidence as to the relative variances of offspring production through male versus female function. In general, however, male reproductive success has been assumed to have the greater variance. Under this assumption, predictions from Gillespie's principle would be very different than those from

Bateman's principle.

The gamete-trading model (Leonard and Lukowiak, 1984, 1985), developed from a comparison of the mating systems of the aglajid opisthobranch, *Navanax inermis* (Cooper) (Leonard and Lukowiak, 1984, 1985) (for discussion of the validity of the genus see Rudman, 1974; Gosliner, 1980), and a serranid fish (Fischer, 1980), is based on the premise that the preferred role for a simultaneous hermaphrodite will be the one that controls fertilization, i.e. is the last to make an irrevocable commitment of gametes. The sexual role that controls fertilization affords the greatest certainty that the investment made will result in zygotes. That is, the preferred role will have the greatest certainty of parenthood. Considered from the standpoint of selection if two reproductive options will have the same mean pay-off but one is less risky than the other, an individual with limited resources to invest will do better to play it safe (Gillespie, 1977; Philippi and Seger, 1989).

Such competing hypotheses as to the source of sexual conflict can be tested in hermaphrodites by finding species for which two of the hypotheses make opposite predictions as to the sexual role that should be favored and then determining which role is in fact preferred. For example, experimental studies designed to test the conflicting hypotheses that in *Navanax inermis*, the male role is preferred as would be expected from Bateman's principle and the egg-trading model (Fischer, 1980; see discussion in Leonard and Lukowiak, 1991), versus the conflicting hypothesis that the female role is preferred (based on the gamete-trading model), indicate a preference for the female role (Leonard and Lukowiak, 1991). In this way, the debate over the factors responsible for sexual conflict can be moved from a theoretical level to an experimental one.

DOES SEXUAL CONFLICT SHAPE MATING SYSTEMS?

The question of the importance of sexual conflict in shaping mating systems could be best answered by comparative studies. That is, we need to decide whether the mating systems observed in simultaneous hermaphrodites conform to what would be expected if sexual conflict were important. A model in the form of a game of strategy, termed Hermaphrodite's Dilemma, has been developed to analyze the situation that arises given a conflict of interest between two simultaneous hermaphrodites in a mating encounter (Leonard, 1990). This model can provide qualitative predictions as to the type of behavior and mating system to be expected under a variety of conditions.

HERMAPHRODITE'S DILEMMA

Briefly, Hermaphrodite's Dilemma is a two-person,

non-zero-sum, conditional game of strategy; the available decisions are: 1) to offer to assume both roles in a mating encounter (the cooperate decision), or 2) to mate only in the preferred sexual role and then desert without allowing the partner to mate in the preferred sexual role (the defect decision). Assuming that both players are, being simultaneous hermaphrodites, prepared to mate in both roles at all times, and that there is an advantage to mating in one role, an individual ought to be willing to assume that role in any and all encounters. Therefore, its decision in a mating encounter is not whether or not to mate in the preferred role, but whether or not it should also mate in the non-preferred role. The model predicts that the best strategy over a wide variety of conditions will be one that combines cooperating (reciprocation) most of the time, with a certain low level of defection (= cheating), which could or could not be contingent on the partner's prior behavior, depending on circumstances [i.e. w, the probability of encountering a partner again, (Axelrod and Hamilton, 1981), and whether the pay-off matrix corresponds to Prisoner's Dilemma or Chicken]. That is, given sexual conflict, the mating system of simultaneous hermaphrodites should be based on reciprocation, with cheating in a species-typical preferred sexual role occurring at a relatively low frequency, and the existence of mechanisms to reduce vulnerability to cheaters (Leonard, 1990).

Furthermore, the model suggests that mechanisms should exist to prevent cheating and/or "punish" cheaters. Although the arguments developed here should apply to all hermaphroditic gastropods, the discussion will focus on the pulmonate and opisthobranch (lumped here as "euthyneuran") gastropods because they offer a wide array of forms of (often bizarre) reproductive behavior [communal parental care (Rose and Hoegh-Guldberg, 1982), hypodermic copulation (Rivest, 1984), chain copulation (*Aplysia* and other species), and elaborate and bizarre forms of courtship (*Helix*, *Limax* and some other stylommatophorans)] that have been little studied. Even the familiar cases (i.e. *Helix*, *Limax*, *Aplysia*) have not been studied in light of modern mating systems theory. The analysis presented here suggests new interpretations of familiar phenomena in gastropod biology and proposes tests of important assumptions and predictions of mating system theory using comparative and experimental studies of these gastropods.

The Hermaphrodite's Dilemma model predicts that where unilateral copulation is the rule, 1) mechanisms for enforcing reciprocation exist [such mechanisms could include explicit alternation of sexual roles, such as that described for *Navanax* (Leonard and Lukowiak, 1984, 1985)], 2) effective reciprocity is achieved by random mating within a small mating group [there is some evidence to suggest that *Aplysia californica* Cooper form small, relatively stable mating clusters (Kupfermann and Carew, 1974)], or 3) willingness to copulate in the less preferred role is maintained by the con-

ditions of the Chicken matrix (Riechert and Hammerstein, 1983). For example, *Lymnaea stagnalis* (L.) are always ready to copulate as females, the preferred role according to the gamete-trading model, but become willing to copulate as males after periods of isolation (v. Duivenboden and ter Maat, 1985). Alternatively, if the mating system were not based on reciprocation, one would expect to see aggressive attempts to force copulation by individuals acting in the favored sexual role, with the victim attempting to avoid copulation and/or to retaliate by assuming the favored role, as could be the case in some leeches (Leonard, unpub. data).

The problem faced by a pair of simultaneous hermaphrodites in a mating encounter differs from that described in the classical Prisoner's Dilemma (Luce and Raiffa, 1957; Davis, 1983) or in the formal Hermaphrodite's Dilemma model (Leonard, 1990) in that a player has information about the decision made by its partner, and in general, one player will have to make the first move. One possible tactic in this situation would be the "Quick-Draw" approach; making the first move and assuming the preferred role, leaving the partner to either assume the non-preferred role or pass up the chance to mate. If the partner agreed to assume the non-preferred role, it would be possible for the initiator to either "cheat" (by leaving without reciprocating) or to reciprocate, by offering to mate in the non-preferred role also. This type of tactic could result in the evolution of either 1) "Hit-and-Run" mating encounters, such as those of some leeches (see above), or 2) insistence by the party of the second part on strong assurance that the initiator will reciprocate, before the party of the second part assumes the non-preferred sexual role. The simultaneous reciprocal copulation found in many nudibranch and stylommatophoran gastropods and clitellate annelids (earthworms and some leeches) could have evolved in this way.

In species in which mating is not (or cannot be) simultaneously reciprocal, an individual pursuing a strategy such as Tit-for-Tat should advertise its willingness to reciprocate, in order to attract potential partners or to avoid rejection by a partner that it has located. Assuming the non-preferred sexual role in the first mating would be a way of accomplishing this. For such a tactic to be successful, there would have to be some protection against "cheating" by the partner. Such tactics appear to be employed as part of a Tit-for-Tat strategy in the mating systems of certain serranid fishes (Fischer, 1980, 1984) and at least one opisthobranch gastropod, *Navanax inermis* (Leonard and Lukowiak, 1984, 1984, 1991).

EUTHYNEURAN GASTROPODS

GENERAL REPRODUCTIVE BIOLOGY (for review see Tompa *et al.*, 1984)

All but a very few species of the euthyneuran (Sub-

classes Opisthobranchia and Pulmonata) gastropods are simultaneously hermaphroditic. Among euthyneuran groups, gonochorism is common only in the opisthobranch order Acochlidioidea (Hadfield and Switzer-Dunlap, 1984; but see Wawra, 1988). While many authors refer to protandry in these taxa, this usually means only that histological investigations show mature sperm present before eggs have matured. For example, in *Limacina*, an opisthobranch considered to be protandrous, copulation appears to be simultaneously reciprocal between mature males (Lalli and Wells, 1978; other examples in Hadfield and Switzer-Dunlap, 1984). This usage has been common in the molluscan literature since at least the end of the last century (e.g. Pelseneer, 1895). Storage of allosperm (and often autosperm) is common and individuals could receive sperm which they will later use to fertilize eggs, long before eggs have been formed in the ovotestis. True sequential hermaphroditism, in which an individual is purely a sperm donor at one stage of its life and only a sperm recipient at another, is rare, if it in fact exists, in euthyneuran snails (Geraerts and Joose, 1984; Hadfield and Switzer-Dunlap, 1984; Tompa, 1984), although it is not uncommon in the prosobranch gastropods. One must be cautious, therefore, in interpreting references to functional protandry (see discussion in Ghiselin, 1965).

Reproduction through true parthenogenesis (probably automitic) has been reported for one stylommatophoran slug (Hoffmann, 1983; see also discussion in Tompa, 1984). Although the capacity for self-fertilization is not uncommon in pulmonates, and apparently exists in a few opisthobranchs (Hadfield and Switzer-Dunlap, 1984), cross-fertilization is the rule and the vast majority of euthyneuran gastropods act as simultaneous hermaphrodites throughout their reproductive lives.

The anatomy of the reproductive system of euthyneuran gastropods can be summarized as very complex, highly diverse, and taxonomically valuable at the species level. As Eberhard (1985) pointed out, these characteristics are indicative of rapid evolution, probably as a result of sexual selection. This, in turn, suggests that these gastropods should be characterized by diverse, complex and fascinating sexual behavior and mating systems. Unfortunately, our understanding of the reproductive biology of these gastropods is very scanty. For most species only the gross anatomy of the genitalia has been described; less is known at the histological level, and there have been relatively few studies that have looked directly at the physiology of various parts of the reproductive tracts. In many cases it is difficult to imagine how the genitalia would look when everted and how the various parts would fit together during copulation (see Reeder, 1986). Also, most of the available information on sexual behavior consists of casual or anecdotal observations.

In terms of sexual behavior, euthyneuran gastropods can be divided into three groups; those in which copulation

is normally simultaneously reciprocal, those in which it is unilateral, and those in which copulation is unilateral and chains of copulating individuals occur commonly. As a broad generalization, simultaneous reciprocal copulation occurs in taxonomic groups in which the penis and common genital aperture (= vaginal pore) are close together on the body, while unilateral copulation is typical of taxonomic groups in which these structures are widely separated. Speaking again very generally, one can say that simultaneous reciprocal copulation is characteristic of the stylommatophoran pulmonates (exceptions include *Stenotrema*, Webb, 1948), whereas the basommatophorans have unilateral, and sometimes chain, copulation (Geraerts and Joose, 1984; Tompa, 1984). Among the opisthobranchs, simultaneous reciprocal copulation (and/or sperm transfer) is found in most of the nudibranchs, notaspideans, saccoglossans and pteropods, whereas unilateral and/or chain copulation (or sperm transfer) is more common in cephalaspideans, anaspideans, pyramidelloideans and acochlidioideans (Hadfield and Switzer-Dunlap, 1984). True copulation is the general mode of sperm transfer in euthyneuran gastropods, but hypodermic insemination and aphyallic spermatophore transfer have evolved several times among opisthobranchs (Hadfield and Switzer-Dunlap, 1984). Some land slugs deposit sperm on the tip of the partner's penis (Gerhardt, 1933; Tompa, 1984). Aphyallic sperm transfer apparently occurs in some basommatophorans (Geraerts and Joose, 1984). In the nudibranch *Aeolidia* sperm are deposited on the outer surface of the female gonopore (Longley and Longley, 1984).

OPPORTUNITY FOR SPERM COMPETITION

SPERM STORAGE

Although the functions of the myriad organs of gastropod reproductive systems are poorly understood, one can say that both opisthobranchs and pulmonates typically have organs for storage of allosperm and there is some evidence that allosperm could require a period of residence in the sperm storage organ before becoming competent to fertilize eggs (Hadfield and Switzer-Dunlap, 1984). The period of residence in the sperm storage organ is uncertain for most species. Hadfield and Switzer-Dunlap (1984) reported that while some opisthobranchs show a close association between copulation and egg laying, with egg laying following copulations at a fairly predictable interval, this is not always the case. Individuals could copulate many times without spawning. One *Navanax inermis*, under laboratory conditions, copulated 24 times in the female role before laying an egg mass (Leonard and Lukowiak, 1985). The record for the number of egg masses laid between copulations is apparently still held by an *Aplysia californica* taken to Woods Hole, Massachusetts, and held in isolation by MacGinitie (1934). That individual laid eggs for more than four months and the

first 15 egg masses were fertile, demonstrating that *Aplysia* can store sperm for at least two and one-half months and that the sperm storage organ can hold enough sperm to fertilize at least 15 egg masses.

In *Navanax*, one isolated individual laid nine egg masses over the course of a month. The first five egg masses developed normally; the last four were inviable. After being allowed to copulate again (once as a female and once as a male) it laid two egg masses. The first, a small inviable one, was laid within 24 hours of copulation and a large viable egg mass was laid 24 hours later. No further eggs were laid although the individual was maintained in the laboratory for another month (Leonard, unpub. data). In one case, a *Navanax* laid a fertile egg mass after 32 days of isolation (Leonard and Lukowiak, 1985). In *Navanax* both the frequency and size of egg masses vary, and this could reflect size of the animal, food availability and/or sperm stores (Leonard, unpub. data). In isolated *Hermisenda crassicornis* (Escholtz), an aeolid nudibranch, Rutowski (1983) reported that some individuals produced as many as eight egg masses that were at least partially fertile. He reported that *Hermisenda* isolated upon collection produced an average of 3.7 ± 2.5 fertile egg masses within 24 days. He reported 20 days as the longest interval between fertile egg masses but did not indicate how long sperm can be stored after a copulation. In that study, sperm-deleted animals laid egg masses approximately three days after receiving an intromission. Another aeolid, *Phestilla melanobranchia* Bergh, can lay an average of 1.5 fertile egg masses per day for two weeks before depleting the sperm received in a single mating (Harris, 1975).

The possibility of self-fertilization complicates analysis of the relationship between egg laying and copulation in both basommatophoran and stylommatophoran pulmonates. In at least two species of the basommatophoran *Lymnaea*, egg laying begins sooner in mated than in isolated individuals (Horstmann, 1955; Boray, 1964; van Duivenboden, 1983, 1984). Horstmann (1955) established that this effect was mediated by the presence of allosperm in the gametolytic gland. However, van Duivenboden (1984) reported *Lymnaea* raised in groups laid fewer eggs than did isolated individuals.

NUMBER OF MATES

There are at least two reports of apparent monogamy in opisthobranchs. Lalli and Wells (1978: 103) concluded, from anatomical evidence, that in the pelagic pteropod *Limacina inflata* (d'Orbigny) a spermatophore is formed from the prostate gland which must be reciprocally transferred to a partner, because "all *L. inflata* of the proper size have either a well-developed prostate gland or a spermatophore received from another individual, but never both structures". Since this species is anatomically protandrous, an individual presumably mates only once in its lifetime. A different type

of monogamy, involving iteroparity, has been reported from aeolid nudibranchs of the genus *Phestilla*. Rudman (1981: 408) reported that for two species from Tanzania, "At a very early stage individuals would pair with another individual of the same species and they would remain nestled together for their whole lifespan, except when egg-laying or feeding." Individual mature *Phestilla* deposit one or two egg masses per day for several weeks (Harris, 1975). However, as Hadfield and Switzer-Dunlap (1984) suggested, in most opisthobranch species individuals probably mate with a number of different individuals over their lifespan.

Tomba (1984) considered that multiple mating is probably the rule for stylommatophorans and cited evidence from *Cepaea nemoralis* (L.) (Murray, 1964) that an average brood is sired by two individuals and that 10-20 spermatophores have been found in an individual. Lind (1988) concluded that sperm competition will often occur in *Helix pomatia* L. under field conditions. Multiple paternity has also been reported for basommatophorans (Mulvey and Vrijenhoek, 1981; Monteiro *et al.*, 1984; Rudolph and Bailey, 1985). In summary, the available information, while scanty, suggests that while various types of monogamy can occur, most euthyneuran gastropods mate with several sexual partners over their lives and that there is considerable potential for sperm competition and multiple paternity of egg masses. Furthermore, the ability to store sperm for long periods of time can create special problems relative to certainty of paternity. Studies of gastropod reproductive anatomy and physiology from the standpoint of sexual selection and/or sexual conflict acting through sperm competition may shed light on both the function of gastropod reproductive tracts and the evolutionary biology and speciation of gastropods.

PREDICTIONS FROM THEORY

As stated above, the Hermaphrodite's Dilemma model predicts that the mating systems of simultaneous hermaphrodites, including gastropods should a) be based on reciprocity and b) involve a detectable level of "cheating" in a favored role. In some euthyneuran gastropods, reciprocity in the mating system is obvious and here the novel prediction is that of "cheating" in a favored role. In other groups reciprocity is not obvious or not known and thus is, in and of itself, a strong prediction of the model. A further problem is to predict which sexual role will be preferred. Bateman's principle and the egg-trading model of hermaphrodite mating systems (Charnov, 1979; Fischer, 1980; see also discussion in Leonard and Lukowiak, 1991) predict that the male role will be preferred except where the male contributes something other than sperm to the mating (i.e. parental care, a nutritional investment, etc.) which makes male parental investment larger than female parental investment. Because one cannot determine which investment is larger *a priori*, I

assume here, for the sake of simplicity, that the egg-trading model consistently predicts a preference for the male role. The gamete-trading model (Leonard and Lukowiak, 1984, 1985) predicts that where the female controls fertilization the mating system will be based on sperm trading. That is, the female role will be preferred in general in euthyneuran gastropods, and that exceptions should be found in those species that lack a gametolytic gland and/or sperm storage. Thus, for most species of euthyneuran gastropod the egg-trading model predicts a preference for the male role and the gamete-trading model predicts a preference for the female roles. Specific predictions and tests of these models are discussed below.

CASE STUDIES OF MATING SYSTEMS

NAVANAX INERMIS AND SPERM-TRADING

There has been some progress in the analysis of sexual conflict in one of the unilaterally copulating opisthobranchs, *Navanax inermis* (Leonard and Lukowiak, 1984, 1985, 1987a, 1991), which has served as the stimulus for development of the gamete-trading and Hermaphrodite's Dilemma models. The mating system of *Navanax* is based on reciprocation in that pairs of individuals actively alternate sexual roles over a series of copulations. This mating system is analogous in many respects to that of the egg-trading serranid fishes (Leonard and Lukowiak, 1984, 1985; Leonard, unpub. data), in that two individuals remain together for a series of copulations but there is no long-term pair bond. The major difference is that in serranids the male role is preferred as predicted by Bateman's principle (Fischer, 1980; Leonard, unpub. data). In *Navanax*, both qualitative observations (Leonard and Lukowiak, 1984, 1985) and experimental tests (Leonard and Lukowiak, 1991) indicate that it is female, rather than the male, sexual role that is preferred. The preference for the female sexual role in *Navanax* has been hypothesized to be a consequence of female control of fertilization (Leonard and Lukowiak, 1984, 1985, 1991).

In *Navanax*, as in most euthyneuran gastropods (Pruvot-Fol, 1961; Tompa, 1984; Geraerts and Joosse, 1984; Hadfield and Switzer-Dunlap, 1984), there is both a sperm storage organ and a gametolytic gland (Rudman, 1974), and ovulation is not tied to copulation. Consequently, sperm transferred to a partner could be "wasted" in that they could be digested rather than stored, and if stored they could or could not be used for fertilization while they (the sperm) are still viable. In the serranids, on the other hand, female courtship displays are closely tied to ovulation and the "male" has reliable information as to the onset and duration of spawning (Fischer, 1980). Therefore, the "male" has greater control over the fate of its gametes than does the "female," and this could be generally true of externally-fertilizing fishes (Alexander and Borgia, 1979). The gamete-trading model

predicts that simultaneous hermaphrodites will prefer the sexual role that offers control of fertilization (Leonard and Lukowiak, 1984, 1985, 1991). Therefore, the female role should be preferred in most euthyneuran gastropods (Leonard and Lukowiak, 1985).

The mating system of *Navanax*, termed sperm-trading, represents a mirror image of the egg-trading system. In *Navanax* a sexual encounter is initiated by an individual tracking down, courting and copulating in the male role (indicating willingness to reciprocate by starting out in the less-preferred sexual role). The available evidence suggests that males maintain intromission until the partner reciprocates (Leonard and Lukowiak, 1984, 1985, 1987a, 1991). This could serve to enforce reciprocation by preventing the partner from mating again as a female before serving as a male to its current partner. The (indirect) evidence that "cheating" occurs in *Navanax* is twofold: 1) reciprocation does not always occur, and 2) the complexity and variability of behavior observed during alternation of sexual roles suggests that cheating attempts may be occurring at this time (Leonard and Lukowiak, 1985, 1987a). In *Navanax*, sperm transfer is not directly observable and at present there is no evidence as to whether or not "subtle cheating" (Trivers, 1971) in the form of failures to transfer sperm during a copulation, or the transfer of substandard quantities of sperm, is occurring.

BIOMPHALARIA AND SPERM-SHARING

Evidence for what could represent a type of "subtle," probably even "victimless" cheating on a sperm-trading mating system, does exist in planorbid basommatophoran pulmonates of the genus *Biomphalaria*. Using genetic markers, Monteiro *et al.* (1984) demonstrated that *Biomphalaria* copulating as males sometimes transfer, to a female partner, sperm that they have received from a previous partner. That is, a snail copulating as a male may inseminate its partner with allosperm instead of, or along with, autosperm. This phenomenon appeared paradoxical at first glance because Bateman's principle can not explain how an individual would benefit by distributing someone else's sperm. The most probable functional explanation of this phenomenon is that it is a form of "cheating" in a sperm-trading mating system, serving to allow a snail to receive more sperm from a partner than it gives up (in autosperm) (Leonard and Lukowiak, 1987b; Monteiro *et al.*, 1987). This prediction was made on the basis of the gamete-trading model and analogy with *Navanax* (Leonard and Lukowiak, 1985, 1987b). This is a strong prediction because although copulation in *Biomphalaria glabrata* (Say), at least, is typically unilateral and reciprocation can occur (Brenner, 1990), the details of the mating system are as yet unknown. That is, it remains to be shown that the mating system is based on reciprocation with courtship performed by the male, and a preference for the female role, as is required for sperm-trading. Sperm-

parcelling, in which only a small quantity of sperm (too little to fill the sperm storage organ) is transferred in a single copulation, is expected to form part of sperm-trading mating systems (Leonard and Lukowiak, 1984, 1985). M.E.B. Valadares-Ribeiro has obtained evidence from studies with genetic markers of sperm-parcelling in *B. tenajophila* (pers. comm. from W. Monteiro). Sperm-parcelling has yet to be demonstrated directly for *Navanax*, although there is some evidence that a single copulation as a female does not completely replenish depleted sperm stores (Leonard, unpub. data, see also above).

OTHER MATING SYSTEMS WITH UNILATERAL COPULATION

To date, *Navanax* is the only species for which repeated alternation of sexual roles in a copulatory bout has been described. Further investigation could show that this is not uncommon in unilaterally copulating gastropods since very little is known about the copulatory behavior of most of them. However, it is clear that repeated alternation is not characteristic of some taxa, e.g. *Lymnaea*, *Aplysia*, etc. There are three types of mating interaction that have been described for these species: single non-reciprocal copulations, a single alternation of sexual roles, and chain copulation.

LYMNAEA AND OTHER BASOMMATOPHORANS

A single alternation of sexual roles commonly occurs in the lymnaeid basommatophorans, *Lymnaea stagnalis* (L.) (Noland and Carriker, 1946; Barraud, 1957; van Duivenboden, 1984) and *Stagnicola elodes* (Say) (Rudolph, 1979a) and perhaps also in the stylommatophoran genus *Partula* (Lipton and Murray, 1979). In contrast, reciprocation does not occur commonly in the planorbid basommatophoran *Bulinus globosus* (Morelet) (Rudolph, 1979b). The mechanisms by which reciprocation is enforced (or cheating prevented) in cases of a single alternation of sexual roles have not been studied in detail. In *S. elodes* and *L. stagnalis*, male sexual behavior has been shown to be induced by copulation as a female (Rudolph, 1979a; v. Duivenboden and ter Maat, 1985) and this could also be the case in *Navanax* (Leonard and Lukowiak, 1991). In *Lymnaea*, courtship is a male behavior and individuals appear to be always willing to copulate as females (van Duivenboden and ter Maat, 1985), which suggests that the female sexual role is preferred. Also, copulatory plugs have been reported in *S. elodes* and *B. globosus* (Rudolph, 1979a, b). That of *S. elodes* probably prevents a second copulation as a female for two to three hours, while in *B. globosus* the copulatory plug is presumed to be ineffective in preventing a second copulation. It would be interesting to know more about the relationship of these plugs, apparently common in basommatophorans (Geraerts and Joosse, 1984), to the mating system.

These instances of apparently unilateral and/or single

reciprocal copulations offer exciting opportunities to test the Hermaphrodite's Dilemma model, because they appear at first glance to contradict the predictions of the model. The first prediction of the model is that of reciprocity. Thus in order to be consistent with the model, the cases of unilateral copulation must actually represent part of a reciprocal interaction [which is not impossible, the single alternation of *Lymnaea stagnalis* could involve a period of hours between the two copulations during which the first male rides on the shell of the first female before she begins to reciprocate (Leonard, v. Duivenboden and ter Maat, unpub. data)] or the unilateral copulations must represent a form of "cheating" obviously derived from a reciprocal mating system. The cases of a single reciprocal copulation are also puzzling. Single reciprocal copulations would be consistent with the predictions of the Hermaphrodite's Dilemma only under Game of Chicken conditions. That is, if there is a preferred role, an individual that has taken that role in the first copulation would have no reason to remain and reciprocate by assuming the less preferred role, unless there were a shortage of other mates available. Because many of the basommatophorans, such as *Lymnaea* and *Physa*, for example, typically occur in dense populations this seems unlikely. Such population densities suggest that Prisoner's Dilemma conditions should be in operation but it is axiomatic (Axelrod and Hamilton, 1981) that under Prisoner's Dilemma, reciprocation (such as Tit-for-Tat) can only evolve where the last move of the interaction is known. Otherwise, each player would benefit by refusing to reciprocate on the last move (see Leonard, 1990). Therefore, the Hermaphrodite's Dilemma model predicts that single reciprocal copulations must involve as yet identified mechanisms for enforcing reciprocation. Specifically, where a gametolytic gland exists, it should be the case that the male is able to prevent the female from leaving before reciprocating by assuming the male role with its former partner. The egg-trading model, on the other hand, would predict that individuals should compete for opportunities to copulate as males.

CHAIN COPULATION

Chain copulation consists of a mating interaction between three or more individuals in which the individual in front acts only as a female, while each middle individual acts both as a male (to the individual in front of it) and as a female (to the individual behind it) while the last individual acts only as a male. This phenomenon has been observed in a variety of euthyneuran gastropods, particularly basommatophoran pulmonates and tectibranch opisthobranchs (see various planorbids, Precht, 1936; Duncan, 1975; Kuma, 1975; Geraerts and Joosse, 1984; Hadfield and Switzer-Dunlap, 1984; Franc, 1986). Chain (and even ring) formation appears possible in virtually all species that normally copulate unilaterally, and some that usually copulate

reciprocally (e.g. *Phyllaplysia taylori* Dall, Beeman, 1970a, b), and is particularly common under crowded laboratory conditions. In some taxa, such as *Aplysia* spp., however, chain copulation appears to occur commonly in the field, and must be regarded as a normal feature of the mating system (P. Fischer, 1869; MacGinitie and MacGinitie, 1968; Ricketts *et al.*, 1968; Kupfermann and Carew, 1974; Leonard, unpub. data). Chain copulation has also been described from laboratory observations for *Acera bullata* Müller (Legendre, 1905). Geldiay (1956) concluded that chain copulation was the rule rather than the exception for Lake District populations of the freshwater limpet, *Ancylus fluviatilis* Müller, where chains of as many as seven individuals have been observed. Wesenberg-Lund (1939) reported for *Lymnaea* that chains of three individuals were not uncommon in the field (see also Crabb, 1927; Noland and Carriker, 1946; Barraud, 1957; v. Duivenboden, 1984) and that the female will next act as male to a nearby individual. There are also reports of simultaneous reciprocal copulation in *Lymnaea* (Klotz, 1889; Crabb, 1927). In other species, chain copulation is probably largely an artifact of laboratory conditions [e.g. *Physa fontinalis* (L.) (Duncan, 1959)] and rare, if it occurs at all, in the field. For example, in *Navanax* chains and/or rings of three or four copulating individuals occur commonly in the laboratory, but are very rare in the field (Leonard and Lukowiak, 1985). Rivest (1984) described group hypodermic copulation in two species of the nudibranch *Palio*, but this appears to be the exception, the rule being simultaneously reciprocal hypodermic copulation.

The Hermaphrodite's Dilemma model predicts that chain copulation should represent an obvious derivative from a reciprocal mating system. The data available in the literature are not adequate to confirm or refute this prediction and further observations are required before we can understand chain copulation as a mating system. In *Lymnaea*, mating interactions typically involve a single alternation of sexual roles between members of a pair (Noland and Carriker, 1946; Barraud, 1957; v. Duivenboden, 1984; v. Duivenboden and ter Maat, 1985; Leonard, v. Duivenboden and ter Maat, unpub. obs.) and it could be the case that chain copulations occur under conditions of high density and could represent "cheating" on a successively reciprocal system. If so, the gamete-trading model predicts that the "cheating" will consist of females avoiding male behavior, whereas the egg-trading model would predict that individuals should compete for opportunities to copulate as males. Another possibility is that mechanisms exist for reciprocation within the chain interaction. That is, individuals in chains could remain in the chain until they have copulated equally often in both roles. Some observations in both *Lymnaea* (Wesenberg-Lund, 1939) and *Aplysia californica* (Leonard and Lukowiak, 1983; Leonard, unpub. data) suggest that, as in *Navanax* (Leonard and Lukowiak, 1987a, 1991), individuals begin to act as males

after acting as females. Also, laboratory observations indicate that chains of copulating *A. californica* can break and reform and individuals can copulate several times before mating activity ceases, with some indication that females (individuals at the front of the chain) tend to act as males to either the animal at the end of the chain or a nearby individual in the subsequent copulation (Leonard and Lukowiak, unpub. data). The data, however, are too scanty to allow us to tell whether individuals alternate sexual roles within chains. In *Aplysia*, courtship is initiated by the individual that will act as a male (Kupfermann and Carew, 1974; Leonard and Lukowiak, 1983) as is the case in *Lymnaea* and *Navanax* but there is as yet no clear evidence that the female is preferred as predicted by the gamete-trading model.

THE STRANGE CASE OF *ARIOLIMAX*: SELF-MULTILATION? HERMAPHRODITES AS "CASTRATING FEMALES"?

Another intriguing observation is the report for a stylommatophoran slug, *Ariolimax*, that "they frequently gnaw off the penis at the close of copulation," (Mead 1943: 675). A certain percentage of large individuals in *Ariolimax* appear to lack completely a penis (Heath, 1916; Mead, 1943), whereas in others it is underdeveloped (Heath, 1916). Heath (1916), having hypothesized that the penis must be lost and then regenerated in this species, collected 200 individuals in an enclosure and after several weeks was able to observe two instances of copulation. He described the courtship process and stated that copulation was unilateral and that in both cases the penis was chewed off as soon as the animals began to draw apart. He indicated that in at least one case the amputation was initiated by the female who was then joined in amputation by the "possessor of the intromittent organ concerned". Upon dissection Heath found that in two of the individuals (the females in the copulations), the amputated penis extended from the genital pore internally to the distal end of the seminal receptacle. Heath found this phenomenon understandably perplexing and offered two possible explanations: 1) that the amputated penis serves as a sperm plug; 2) that the behavior is an artifact of disturbance by the observer and has evolved as a means of rapid separation when escape is necessary. Because the amputation process took over 10 min (Heath, 1916), it seems unlikely that it is an effective defense against predators but it could serve to prevent dessication. Heath mentioned that copulation is nocturnal and that intromission had lasted several hours before the animals began to separate so it may be the case that copulations starting late in the night might create a risk of dessication in the morning sun unless there was a way of rapidly terminating them. Sexual conflict theory can add 1) the possibility that the function of the sperm plug is more to prevent the mate from acting as a female again (keep other sperm out) than to prevent loss of sperm, and/or 2) the suggestion that in these her-

maphrodites an individual that amputated the penis of its mate could increase its own reproductive success as a male by decreasing the number of effective male rivals.

The Hermaphrodite's Dilemma model would predict that this behavior, if it is not merely a defense mechanism against danger of dessication or the like, must be a means of enforcing reciprocation or "cheating" on a reciprocal mating system. For example, if as predicted by the gamete-trading model, the female role is preferred it could be the case that once an individual has accumulated enough allosperm to fill its own sperm storage organ, it could not have any "reason" to mate as a male and could amputate its own penis, leaving it as a sperm plug to prevent its mate from receiving more sperm before egg-laying, thereby insuring paternity. It is barely conceivable that an individual could be able to regenerate its penis in time to get to use it in reciprocal mating interactions in order to obtain a new load of sperm after using the previous batch. In any case, the gamete-trading model predicts that *Ariolimax* which lack a penis should remain willing to copulate as females whereas the egg-trading model would predict that, because hermaphrodites should copulate as females in order to get an opportunity to copulate as males, that an individual lacking a penis ought to be unwilling to copulate as a female. Similarly, if the goal of copulating as a male is getting an opportunity to be female, as is predicted for euthyneuran gastropods by the gamete-trading hypothesis, then *Ariolimax* should be reluctant to act as a male to an individual that lacks a penis. The egg-trading model on the other hand predicts that an individual copulating as a male should not be fussy and should accept a mate with or without a penis of its own. Therefore, both gamete-trading and egg-trading predict that individuals lacking a penis will be unlikely to be involved in copulations but the egg-trading model predicts that that will be due to "coyness" of the amputee, whereas the gamete-trading model predicts that the individual lacking a penis will be unattractive or rejected as a (female) mate.

The copulation of these common banana slugs of the northwest coast of the United States seems to demand further attention. We need to know: 1) whether this amputation is a defensive response or whether it occurs as a normal part of the sexual behavior; 2) how commonly this amputation occurs; 3) who amputates the penis of whom; and 4) whether this amputation occurs after an individual's first copulation or only in older individuals who may have mated with several partners. We also need to know how often these animals copulate over their life-span, and/or between egg-layings in order to understand the significance of this. Perhaps these slugs are effectively monogamous, at least as males, each individual mating once upon attaining adulthood and losing its penis in the process, with occasional individuals surviving long enough to regenerate the penis and copulate as males a second time? Its amazing how little we know about such

common and conspicuous animals.

SIMULTANEOUSLY RECIPROCAL COPULATION

STYLOMMATOPHORANS

Both Hyman (1967) and Franc (1968) made the interesting generalization that basommatophorans have unilateral copulation associated with a short, simple courtship performed by the individual that will act as the male, whereas stylommatophorans have simultaneously reciprocal copulation preceded by lengthy, elaborate, and often bizarre courtship behavior. The usual explanation of this phenomenon has been mechanistic; i.e. that the behavior serves to facilitate coordination between the partners to allow simultaneous reciprocal intromission, and most of the experimental work has focused on that aspect of the behavior (i.e. *Helix*, Jepsen, 1976; Lind, 1976; Chung, 1986; Adamo and Chase, 1988; Giusti and Andreini, 1988). However, because many opisthobranchs, particularly nudibranchs, have simultaneous reciprocal copulation without lengthy or notably peculiar courtship behavior (Hadfield and Switzer-Dunlap, 1984; Leonard, unpub. data; see also aeolids below), it is difficult to argue that simultaneous reciprocal copulation must be accompanied in evolution by such bizarre mechanisms as the love-dart of *Helix*, the ingestion of caudal mucous globules, the aerial performance of *Limax maximus* (L.), etc. (see Hyman, 1967; Franc, 1968; Tompa, 1980). An obvious functional or adaptive explanation is that these elaborate courtship behaviors have evolved through sexual conflict.

Specifically, the Hermaphrodite's Dilemma model predicts that they all serve to prevent "cheating" on the reciprocal mating system, which should take (according to the gamete-trading model) the form of individuals attempting to act as females, receiving sperm, without offering any of their own. Both Meisenheimer (1907) and Lind (1976) reported that in *Helix*, that an individual (A) that inserts its penis into the vagina of its partner (B) will immediately withdraw its penis unless B simultaneously inserts its (B's) penis into A's vagina (but see Chung, 1987). This is consistent with the idea that courtship serves to prevent an individual from acting only as a male. Similar reluctance to act as a male before the partner does should be seen in other species with elaborate courtship and simultaneous reciprocal copulation. The courtship therefore, should enforce reciprocity, specifically by preventing individuals from acting only as females. An egg-trading model, based on Bateman's principle would predict the opposite; that is the courtship serves to enforce reciprocity by preventing individuals from "cheating" by acting only in the male role. One would predict therefore that in these elaborate courtships there should be evidence that individuals are 1) coy as females, refusing to allow intromission until they have an opportunity to act as a male; and 2) eager as males,

competing with each other for the first intromission. Charnov (1979) suggested that such "complicated precopulatory displays" should serve (under Bateman's principle) to induce the partner to use the sperm received to fertilize eggs. These hypotheses should be testable by experimental and comparative studies of common species of stylomatophorans.

AEOLIDS

While Lind (1976) reported that unilateral copulations were rare in *Helix*, Rutowski (1983) found that 49% of all copulations in *Hermisenda* were non-reciprocal. Rutowski (1983) discussed this phenomenon in terms of sexual conflict and Charnov's (1979) prediction, considering the possibility that the failure of one individual to intromit after evertng its penis was the result of an effort by its partner either to deflect the penis of its partner or to give sperm quickly without receiving any. That is, that one individual was attempting to "cheat" by mating only in the male role. He concluded that this was unlikely because many of the "missed individuals" were sperm-depleted and would have benefited from receiving sperm.

If, as the gamete-trading model (Leonard and Lukowiak, 1984, 1985) predicts, the female sexual role is preferred, the "cheater" in a unilateral copulation would be the individual receiving but not giving sperm. These unilateral intromissions in *Hermisenda* could represent "cheating" by "deliberately" missing the target. However, since Rutowski (1983) reported that sperm was ejaculated into the water as a result of these "missed" intromissions this seems improbable. It seems very unlikely that emission of sperm into the water would be more adaptive than transferring it to a partner, especially because Rutowski's (1985) observation that *Hermisenda* ingest any sperm left on the gonopore suggests that the caloric content of sperm is not trivial. I agree, therefore, with Rutowski's conclusion that the high frequency (49%) of copulations in which only one individual achieves intromission is probably a consequence of whatever factors have selected for extremely rapid copulation in this species. However, on the assumption that the female sexual role is preferred in *Hermisenda*, I suggest that cheating was represented in Rutowski's observations by those copulations (5% of the total) which were unilateral because only one individual everted its penis, because in these cases individuals received sperm without giving any in return (or wasting any). A more detailed study of the mating behavior of *Hermisenda* as a function of the sperm stores of the interacting individuals might serve to test this possibility. In particular, Rutowski's observation that most of the "missed individuals" in only semi-successful reciprocal copulation attempts were sperm-depleted is intriguing. One would like to know if sperm-depleted animals behave differently during mating encounters, and/or if their depleted status is detectable by partners who then treat them differently. One would expect that, if there

is any difference, sperm-depleted individuals ought to be both more willing to receive sperm, and more attractive as female partners, than individuals with full sperm stores.

Observations from another aeolid, *Aeolidia papillosa* Bergh, are also suggestive of cheating in a mating system based on reciprocation. The sexual behavior of *Aeolidia* is very similar to that of *Hermisenda* (Longley and Longley, 1982, 1984); encounters are very brief and usually simultaneously reciprocal. However, in *Aeolidia* there is no copulation, sperm packets are deposited on the partner's gonopore (Longley and Longley, 1984). The Longleys observed one individual which copulated repeatedly (over a period of days) without producing sperm packets. These authors also reported that the quantity of sperm transferred in a copulation was determined by both 1) the duration of the copulation, which is correlated with the size of the smaller partner, and 2) the rate of sperm transfer, which is related to the number of autosperm remaining in the ampulla. This raises the possibility that *Aeolidia* could engage in what Trivers (1971) termed "subtle cheating." That is, an *Aeolidia* could cheat by engaging in a reciprocal mating when it has relatively few autosperms available, and thereby receive more sperm than it gives to its partner. Beeman (1970a) also observed instances in which only one member of a pair of reciprocally copulating *Phyllaplysia taylori* Dall transferred sperm to its partner, since the other's ampulla was empty.

Observations on many species with simultaneous reciprocal copulation mention that unilateral copulations sometimes occur (e.g. *Helix*: Herzberg and Herzberg, 1962; Lind, 1976; opisthobranchs: Hadfield and Switzer-Dunlap, 1984; including *Aeolidia papillosa*: Longley and Longley, 1984; *Hermisenda crassicornis*: Longley and Longley, 1982; Rutowski, 1983; *Melilbe*: Agersborg, 1922) which could be considered "cheating." In summary, there is some evidence that "cheating" can occur occasionally in species with simultaneous reciprocal copulation, in the form of unilateral copulations and/or "subtle cheating." However, from the available evidence one cannot say with confidence that "cheating" does or does not occur in these species. The evidence does suggest that studies directed to the analysis of sexual conflict in this group would be very rewarding.

DISCUSSION

The review and analysis presented here suggest that 1) gastropods offer a broad array of reproductive phenomena that require explanation in terms of mating systems theory; 2) Hermaphrodite's Dilemma model makes nontrivial predictions about the mating systems of hermaphroditic gastropods that may serve to test the model; 3) for many euthyneuran gastropods the egg-trading and gamete-trading models make opposing predictions, making this group a useful means of

distinguishing between the two models. While experimental studies can be used to determine the preferred sexual role for a given species (Leonard and Lukowiak, 1991), comparative studies can also be useful here. Review of the range of mating systems found in simultaneously hermaphroditic serranid fishes (Leonard, unpub. data) provides strong support for the existence of sexual conflict since it provides confirmation of the hypothesis that the male sexual role is preferred in these simultaneous hermaphrodites, as assumed by Fischer (1980, 1984) and predicted by Charnov (1979). The evidence for this is twofold. First, in all species studied, "cheating," on a reciprocal mating system, whether as streaking or as extra-pair spawning in the monogamous *Serranus tigrinus* (Bloch) (Pressley, 1981), is a male behavior; cheaters "cheat" in order to fertilize someone else's eggs, not to get their own eggs fertilized. Second, where mates become a defensible resource, large, dominant individuals become male (e.g. *S. fasciatus* (Jenyns) and *S. baldwini* (Evermann and Marsh) (Petersen, 1990). In serranids, then, the harem-based mating systems are exceptions that prove the egg-trading rule. These simultaneously hermaphroditic fish provide strong evidence that sexual conflict both exists and is important in shaping mating systems. However, both the gamete-trading and the egg-trading models predict (indeed the gamete-trading model assumes) that serranids prefer the male role so that the serranids do not allow us to distinguish between the egg-trading and gamete-trading models (contrary to Fischer, 1987). The gastropods therefore, offer an exciting opportunity not only to test the assumption that sexual conflict exists but also to distinguish between models based on different assumptions about the source of sexual conflict. The variety of reproductive behavior and physiology found within the gastropods should allow us to identify species that can be used to test hypotheses about the relative importance of energy investment, mating time and control of fertilization in sexual conflict.

In this paper I have attempted to demonstrate that analysis in terms of sexual conflict makes specific predictions about gastropod mating systems that may allow us to elucidate the adaptive significance of many bizarre phenomena in gastropod reproductive biology. The available literature on gastropod sexual behavior suggests a number of interesting test cases for the Hermaphrodite's Dilemma model, but does not, in itself, provide sufficient data to test the model. The chief difficulty interpreting the available information on gastropod reproduction in terms of sexual conflict or sexual selection is that one can seldom determine from the available descriptions which types of behavior represent the rule and which the exceptions. Where the initial studies were not informed by mating systems theory or selection thinking, crucial information is apt to be lacking, even when there have been numerous detailed studies of the behavior, as in *Helix* (Leonard, unpub. data). Mating systems theory

has a lot to offer to the study of euthyneuran gastropods and euthyneuran gastropods have a lot to offer to the study of mating systems theory.

ACKNOWLEDGMENTS

The author was supported by grants from the NIMH and NSF during part of the period of manuscript preparation.

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Substratum associations of natural populations of Iceland Scallops, *Chlamys islandica* Müller 1776, on the northeastern Grand Bank of Newfoundland

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Abstract. Live, Iceland scallops, *Chlamys islandica*, were enumerated and their occurrences assigned to substratum coarseness grades along five photographic transects (8-10 km in length) covering areas of the northeastern Grand Bank of Newfoundland. Scallops were disproportionately (53-94%) associated with the coarsest grade of substratum comprising dense gravel-cobble (80-100% by area). Overall, scallops were uncommon to rare on predominantly sand substrata. Average densities of scallops per photograph (5.4 m²) ranged from 0.5 to 13.8 in cobble fields and from 0.02 to 1.7 on open sand.

It is hypothesized that Iceland scallops on the northeastern Grand Bank are aggregated on coarse substrata because of a strong propensity towards byssal attachment at all post-larval life history stages. A survey of substratum associations of extant species of *Chlamys* reveals that, with few exceptions, association with coarse substrata is common within the genus.

The Iceland scallop *Chlamys islandica* Müller, 1776, is a subarctic-boreal species, extending from Hudson Strait, N.W.T., south to the Massachusetts region (Lubinsky, 1980). Its bathymetric range extends to about 180 m and over the expansive Newfoundland continental shelf, it can occur at commercial densities (Naidu and Cahill, 1989). Apart from mostly anecdotal accounts, very little is known about substratum associations of natural populations of this species and the family Pectinidae in general. To date, research on substrata associations has focused on the settlement and growth of pectinid spat on artificial collectors within an overall aquaculture context. While it is generally known that pectinid spat will settle on substrata such as algae, hydrozoans, bryozoans and various artificial surfaces (Fraser, 1983), the natural settlement substrata for *C. islandica* are unknown (Wallace, 1982). Subsequent to spat settlement, it is believed that juvenile scallops display an overall movement from primary settlement substrata to substrata where they will reside during juvenile and adult stages. In the case of *C. islandica*, this could take place about one year after settlement when they have attained a shell height of >5 mm (Wallace, 1982).

Based on the contents of scallop dredges, Naidu (1988) found that Newfoundland populations of Iceland scallops are found normally at depths greater than 55 m, usually on hard bottom of variable substratum composition including mixtures of sand, gravel, shell fragments, rocks and boulders. Dense, commercial concentrations of Iceland scallops are known to occur on St. Pierre Bank (Newfoundland Grand Banks) (Naidu and Cahill, 1989) in areas with sediments

characterized as Sable Is. gravel, which is a mixture of gravel and < 10% sand (Fader *et al.*, 1982). Most of the specimens of North American continental shelf *Chlamys islandica* in the collection of the National Marine Fisheries Service (Woods Hole) were collected from coarse substrata comprising gravel, sand-gravel, till and sand (Theroux and Wigley, 1983). Wiborg (1962, cited in Vahl and Clausen 1980) stated that *C. islandica* lives on coarse sediments or on hard bottom. In a shallow water study in the Gulf of St. Lawrence, Jalbert *et al.* (1989) determined that *C. islandica* occurred most frequently on coarse substrata, primarily cobble and gravel.

The objective of this investigation was to identify natural substratum associations in offshore populations of Iceland scallops from the Newfoundland Grand Bank. Based on a series of photographic transects across areas of the northeastern Grand Bank, spatial distribution patterns of the mega-fauna and major substratum types (coarseness grades) were described (Schneider *et al.*, 1987). The scope of this paper is threefold. First, Iceland scallop-substrata associations are documented for the northeastern Grand Bank. This is followed by an assessment of physical and behavioural mechanisms which could potentially influence these associations. Finally, observed patterns of substrata association in *Chlamys islandica* are compared with existing information on substrata and habitats occupied by extant *Chlamys* spp.

STUDY AREA

The Grand Banks of Newfoundland represents the most extensive shallow (< 200 m) feature on the continental

shelf of eastern Canada and is comprised of a series of major banks: St. Pierre, Green, Whale and Grand. The study area is situated on the northeastern edge of the Grand Bank of Newfoundland in the vicinity of the Hibernia oilfield (Fig. 1). Water depths range from 70 to 100 m. The most complete description of this area in terms of the surficial sediments and seabed processes is found in Barrie *et al.* (1984). Sedimentary cover typically consists of reworked sand and gravel deposits, generally < 2 m thick. Bedforms include sand ripples (≤ 1 m wide), sand megaripples (5-10 m wide), sand ribbons (100 m to 1 km wide) and sand ridges (generally > 3 km in width). Coarse sediment is incorporated into the sand, in places. Coarse sediment consists of gravel and cobble-sized clasts (Grand Bank Gravel) which are believed to represent Pleistocene glacial deposits which have been reworked subsequently into coastal environments by an early Holocene marine transgression as a result of a eustatic rise in sea level. Overall, sedimentary bedforms on the Grand Banks are believed to be dynamic and are being reworked by unidirectional storm-driven currents, ocean currents and extreme waves.

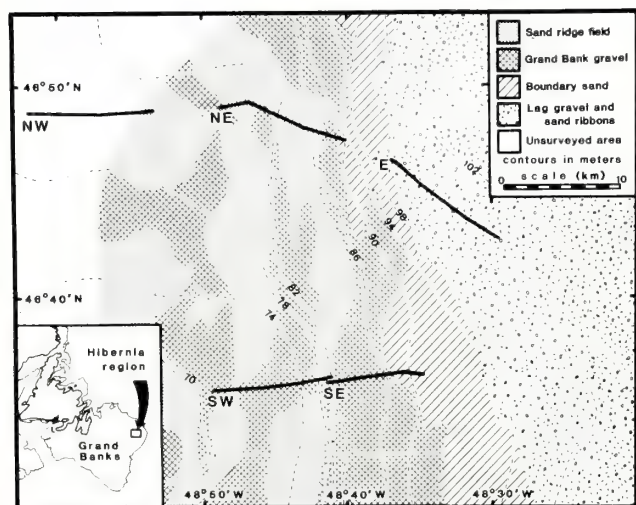


Fig. 1. Location of photographic transects on the northeastern Grand Bank.

METHODS

Five photographic transects, ranging in length from 8 to 10 km, were conducted across the northeastern edge of the Grand Bank (Fig. 1). Colour 35 mm slide photographs were taken at 10 s intervals with the BRUTIV system (Vilks, 1984) which consists of a sled-mounted camera (aligned vertically) towed 3 m above the seabed. Technical details are provided in Schneider *et al.* (1987). The area defined by each photograph was 5.4 m².

Each frame (slide) was examined under low magnification (X16) and classified into one of six substratum categories

based on a gross classification scheme of sediment texture and estimated areal coverage:

- 1- pure sand;
- 2- sand with scattered cobble;
- 3- scattered gravel on sand;
- 4- sand with cobble and shell;
- 5- 50 to 80% cobble and gravel;
- 6- > 80% cobble and gravel.

Sand and gravel were distinguished on the basis of textural differences. While gravel is defined as particles with diameter > 5 mm (ASTM, 1988), much of the sediment in categories 5 and 6 were dominated by large-sized rocks (> 20 mm diameter) and are referred to as cobble.

All identifiable Iceland scallops were enumerated by superimposing a 5 X 5 grid over each slide and recording the number of organisms in each cell. Analyses were carried out on the sum of counts in each slide. Megafauna less than about 2 cm in shell height were visible, but generally could not be identified to the species level. Scallops were classified as live if they were in normal life position (epifaunal) with normal colouration (i.e. not bleached). While there could be a tendency to overestimate the abundance of live scallops by inclusion of articulated, collapsed cluckers (*sensu* Naidu, 1988), there were probably instances where live, "bleached-looking" scallops were classified as dead. All identifiable scallops displayed a sculpture of coarse ribs and had unequal hinge "ears," features which distinguish the Iceland scallop from the sea scallop *Placopecten magellanicus* (Gmelin, 1791). The only scallop reported from the northeastern Grand Bank has been the Iceland scallop (Mobil, 1985).

For each transect, the frequency distributions of live scallops, by substratum type, were tested statistically for significant departures from random distributions based on the proportion of available substratum types (G-test for goodness of fit, Sokal and Rohlf, 1981). It was assumed that scallops would be distributed randomly at similar densities for all substratum types.

RESULTS

The bedforms which the photographic transects crossed are shown in figure 1. Transect Northwest (NW), which over most of its length traversed previously unsurveyed territory, crossed a sand ridge field with areas of scattered cobble and gravel. Transect Northeast (NE) intersected two sand ridge fields with minor amounts of cobble, gravel and shell. Transect East (E) crossed sand ribbons developed on top of a lag gravel. Transect Southwest (SW) crossed a gravel and cobble field (Grand Bank gravel) with a regular alternation of sand and cobble and Transect Southeast (SE) crossed a gravel and cobble field (Grand Bank gravel) which intersected a sand field (boundary sand) at its eastern end.

Substratum composition varied between transects (Fig.

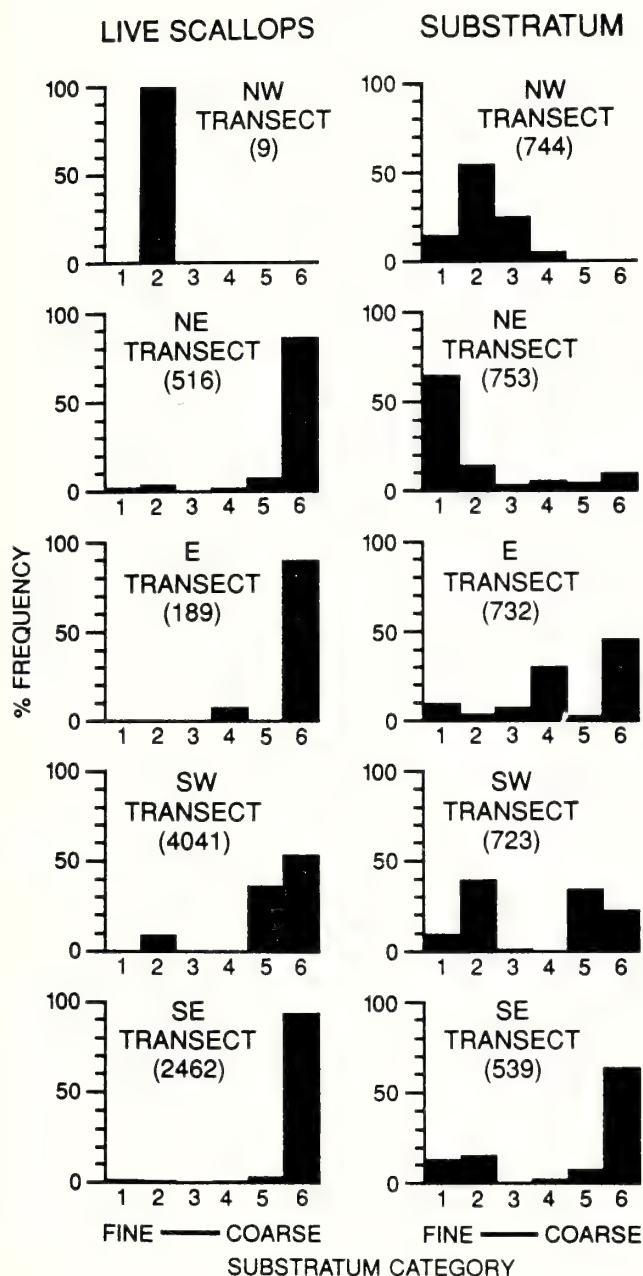


Fig. 2. Frequencies of occurrence of (i) live Iceland scallops by substratum category and (ii) substratum categories along photographic transects. Numbers in parentheses refer to total number of enumerated live scallops (left column) and photographs examined (right column) along each transect.

2). Transects E and SE had a high percentage (50-60%) occurrence of predominantly coarse substrata, comprised of gravel and cobble (some shell), whereas Transects NE and NW had high occurrences of primarily sand substrata (> 60% in both cases). Along Transect SW there was more or less equal representation of fine and coarse substrata.

Along those transects which included the two coarsest substratum categories, scallops were aggregated on coarse

substrata (i.e. gravel and cobble) (Fig. 2). Along Transects NE, E and SE, greater than 80% of the total number of live scallops occurred on the coarsest substratum (category #6). This was in spite of the fact that predominantly fine substrata occurred at frequencies ranging from 15 to 80% along these transects. In particular, along Transect NE where the pure sand substratum category (#1) had a frequency of occurrence of 65%, approximately 85% of the scallops occurred on cobble substrata which had a frequency of occurrence of only 10%. Along Transect SW, approximately 10% of the scallops were counted from substrata comprised predominantly of sand and scattered cobble (Fig. 2). It is noted that this occurred along the transect with the highest densities of live scallops (Fig. 3). Frequencies of scallops along these transects showed a highly significant deviation from a random distribution across all substratum types (G-test statistic range: 176-2869, $p < 0.01$). Transect NW crossed a sand ridge field with areas of scattered gravel. Along this entire 10 km transect, represented by 744 photographs, only 9 live scallops were counted.

There was considerable variation in the substratum-specific densities of live scallops between transects (Fig. 3). Average densities on the coarsest substratum (> 80% cobble and gravel) ranged from 0.5 to 13.8 scallops per photograph (0.09 to 2.5 scallops/m²) while average densities on the next coarsest substratum (50% to 80% cobble and gravel) ranged from 0.1 to 5.7 scallops per photograph (0.02 to 1 scallop/m²). The highest densities on fine substrata occurred on Transect SW with an average density of 1.7 scallops per photograph (0.3 scallops/m²) on a substratum consisting primarily of open sand with scattered cobble. It was noted previously that this transect displayed the highest densities of scallops on most substratum categories. On the remaining transects, densities ranged from 0.02 to 0.4 scallops per photograph (0.003 to 0.07 scallops/m²) on the two finest substratum categories.

DISCUSSION

It is obvious that there is a strong association between Iceland scallops and coarse substrata (gravel, cobble) on the northeastern Grand Bank. At the outset, there are several explanations which could account for the observed aggregated distribution of scallops on coarse substrata. These include (1) substratum-specific predation pressure and (2) behavioural and physical mechanisms maintaining scallop-substratum associations.

PREDATORS

The underlying premise of substratum-specific predation pressure is that scallops suffer heavy mortality from predators after movement of juveniles or adults onto fine

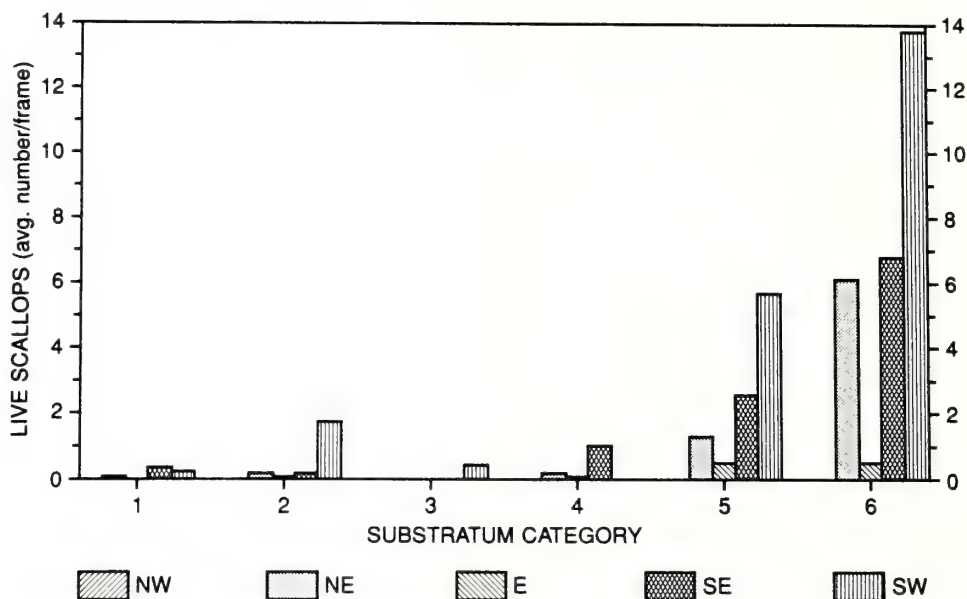


Fig. 3. Average densities of live Iceland scallops by substratum type along photographic transects.

substrata. As mentioned previously, evidence to date on peccinid spat substratum preference rules out primary settlement on fine substrata (i.e. sand). While disproportionate predation pressure due to predator-substrata associations cannot be ruled out as a contributing factor, we believe that predators on the northeastern Grand Bank are not responsible for the skewed distribution of scallops on coarse vs. fine substrata.

Potential predators of Iceland scallops on the Grand Banks are listed in Table 1. Of these, only the pleuronectids, American plaice (*Hippoglossoides platessoides* Fabricius, 1780) and yellowtail flounder (*Limanda ferruginea* Storer, 1839), are known predators of Iceland scallops on the Grand Banks (Pitt, 1976; Naidu and Meron, 1986) and other continental shelf regions (Langton and Bowman, 1981). Naidu and Meron (1986) determined that Iceland scallops occurred in plaice stomachs with a frequency of 22% on St. Pierre Bank. They found that Iceland scallops were susceptible to predation until the age of five years, at which point they achieved a size refuge which was a function of predator mouth gape. While probably not accounting for the rarity of scallops on fine substrata on the northeastern Grand Bank, American plaice would enhance the contrast in distribution of scallops between coarse and fine substrata through predation on these relatively rare occurring individuals on fine substrata.

Other large predatory fish such as Atlantic cod (*Gadus morhua* Linnaeus, 1758) are not known to be associated with any particular substratum type and are typical opportunistic feeders. Examination of cod stomach contents from the Grand Banks reveals a very low incidence of Iceland scallops (G. Lilly, pers. comm.).

Little is known about invertebrate predators of Iceland scallops. The Buccinidae and the Naticidae are probably the

Table 1. Potential predators of the Iceland scallop, *Chlamys islandica*, on the northeastern Grand Bank of Newfoundland.

Predator ¹	Substratum	
	Fine	Coarse
MOLLUSCA		
Buccinidae	P	P
Naticidae	P*	
CRUSTACEA		
Majidae- includes <i>Hyas</i> spp., and <i>Chionocetes opilio</i>	P	P
ECHINODERMATA		
Asteroidea		
<i>Asterias vulgaris</i>		P*
<i>Leptasterias</i> sp.		P*
CHORDATA		
Rajidae	P*	
Gadidae	P	P
Zoarcidae	P	P
Cottidae	P	P
Pleuronectidae	K*	
<i>Pseudopleuronectes americanus</i>		
<i>Limanda ferruginea</i>		

¹ -Identified along photographic transects (Schneider *et al.*, 1987)

* -Typical substratum association

P-potential predator

K-known predators (Langton and Bowman, 1981; Naidu and Meron, 1986)

two major predatory gastropod groups on the Grand Banks. Species lists are incomplete for the study area and ecological relationships are poorly documented. However, from studies conducted in coastal areas, it is known that adult *Buccinum undatum* (Linnaeus, 1758) can be attracted over considerable distances (> 50 m/day) in search of bivalves which are their primary prey, on substrata including sand, mud and rock

(Himmelman, 1988; Jalbert *et al.*, 1989). Buccinid snails were common over all photographic transects and substratum types, although never abundant. The maximum average density for a transect was 0.4 snails/photograph (0.07 snails/m²) (Schneider *et al.*, 1987). Although uncommon in photographs, naticid gastropods were observed along the study transects on sand substrata. Most species of Naticidae are relatively stenotypic and prefer sand or muddy substrata (Golikov and Sirenko, 1988). If these gastropods were exerting a heavy mortality on scallops, one would expect to find a surface accumulation of empty scallop shells; this was not observed. Alternatively, naticids could be preying upon small scallops in the size range below the limits of resolution in photographs (i.e. < 2 cm).

Crabs are known predators of various scallop species (Elner and Jamieson, 1979; Lake *et al.*, 1987). While potentially important predators of scallops such as majid crabs (*Hyas* spp. and *Chionocetes opilio* O. Fabricius, 1780) were common over most transects, they were not restricted to a particular substratum (Schneider *et al.*, 1987). The extent of predation by various echinoderms (e.g. Asteroidea) on scallops is unknown, however, it is noted that asteroids were primarily associated with the coarsest substrata on the transects (Schneider *et al.*, 1987) and, therefore, would not be expected to cause excessive mortalities on sand substrata. In conclusion, it is considered unlikely that Iceland scallops are concentrated on coarse substrata due to differential survivorship from intense predation pressure on fine substrata.

BEHAVIOURAL AND PHYSICAL MECHANISMS MAINTAINING SCALLOP-SUBSTRATUM ASSOCIATIONS

The most plausible explanation for the association of Iceland scallops with coarse substrata is the propensity, at all life history stages, towards byssal attachment to a stable substratum. It is known that a high percentage of individuals in a population of Iceland scallops are attached to the substratum by the byssus at any given time. Frequencies of 76% (laboratory) and 97% (field) byssally attached adult scallops have been reported by Naidu and Meron (1986) and Vahl and Clausen (1980), respectively. From diving observations in West Greenland, Pedersen (1989) reported that Iceland scallops were attached to the substratum by the byssus, large scallops were attached directly to the substratum while small scallops were attached to larger scallops or empty shells. Vahl and Clausen (1980) postulate that because *Chlamys islandica* cannot recess on coarse sediments, it remains in danger of being swept away by currents and because *C. islandica* tends to occur in habitats with strong currents (Wiborg, 1962 *vide* Vahl and Clausen, 1980) byssal attachment remains necessary at all sizes.

An important aspect in assessing the importance of water movements in shaping the distribution of scallops is

the current speed required to dislodge byssally attached scallops. Gruffydd (1976) determined "wash-away" velocities for Iceland scallops which ranged from 21 cm/s for 10-20 mm individuals to 26 cm/s for 65-70 mm individuals. For the northeastern Grand Bank of Newfoundland, Barrie *et al.* (1984) hypothesized that periodic, high unidirectional flow velocities (> 50 cm/s) occur, possibly every year to every few years at depths less than 110 m. Under these extreme flow conditions, the coarsest sediments become mobile and move as bedforms. At other times, maximum tidal current velocities (15 cm/s, Mobil, 1985) in the study area are less than those required to "wash-away" scallops. Therefore, within the study area, scallops are probably washed away infrequently although major storms would have the capability of dislodging large numbers of scallops. At present, the extent of the impact of such extreme events is unknown.

Swimming activity would make *Chlamys islandica* susceptible to being "washed-away" from preferred substrata. Gruffydd (1976) determined that all sizes of Iceland scallops, and particularly medium-sized (30-40 mm shell height) individuals, displayed a tendency to swim. However, Vahl and Clausen (1980) considered swimming activity to be a relatively rare phenomenon, with individual scallops making, on average, a swimming excursion every 31 days. This is in spite of the fact that byssus production is a minor item in the energy budget of *C. islandica* (Vahl and Clausen, 1980). There are limited data regarding conditions which initiate the swimming response. While Gruffydd's (1976) experiments showed that the swimming response was strongest at the fastest current speed (15 cm/s), Vahl and Clausen (1980) determined the flight reaction to be less evident during periods when current speeds were strong (about 50 cm/s) and speculated that this was probably due to the high risk to scallops associated with being carried to unsuitable habitats in strong currents. Byssal attachment rate of adult *C. tehuelcha* (d'Orbigny, 1835) increased dramatically over relatively small changes in current velocity, from 65% attachment at 6.6 cm/s to 90% at 8.3 cm/s (Ciocco *et al.*, 1983 *vide* Orensanz *et al.*, in press). Patterns of swimming behaviour in *C. islandica* may be determined by a combination of factors including habitat type and critical current velocity (in terms of initiating the swimming response) specific to these habitat types.

SUBSTRATA AND HABITATS OCCUPIED BY EXTANT *CHLAMYS* SPP.

In the evolution of the Bivalvia, neotenus retention of the byssus was an adaptive break-through in terms of physical stabilization, giving rise to an invasion of new habitats by epifaunal species (Stanley, 1972). The oldest (Triassic) pectinids are of the adult-byssate *Chlamys* type (Triassic) while the emergence of post-Triassic free-living pectinids evolved from byssate forms (Stanley, 1972).

Table 2. Approximate maximum sizes (shell height) and substrata and depths occupied by extant *Chlamys* spp.¹

	Shell Height (mm)	Habitat Substratum	Depth ²	Source
NORTHWEST ATLANTIC				
<i>Chlamys islandica</i> (Müller, 1776)	100	gravelly sand, shell rock	offshore (to 220 m)	this study; Theroux and Wigley, 1983; Naidu and Cahill, 1989
SOUTHWEST ATLANTIC				
<i>C. benedicti</i> (Verrill and Bush, 1897)	13	?	?	Abbott, 1974
<i>C. mildredae</i> (Bayer, 1943)	38	undersides of rocks	inshore (upper subtidal)	Abbott, 1974
<i>C. sentis</i> (Reeve, 1853)	38	undersides of rocks	inshore (< 15 m)	Abbott, 1974; Rehder, 1981
<i>C. ornata</i> (Lamarck, 1819)	40	undersides of rocks	inshore (to 4 m)	Abbott and Dance, 1986
<i>C. imbricata</i> (Gmelin, 1791)	44	undersides of rocks	inshore (< 6 m)	Abbott, 1974
<i>C. multisquamata</i> (Dunker, 1864)	59	rock crevices	inshore/offshore (6-56 m)	Abbott, 1974
<i>C. patagonica</i> (King and Broderip, 1832)	79	consolidated sand, shell	offshore (to 300 m)	Orensanz <i>et al.</i> , in press; O. Iribarne, pers. comm.
<i>C. tehuelcha</i> (d'Orbigny, 1835)	100	consolidated sand; shell- gravel, rocky bottoms	inshore/offshore (< 60 m)	Orensanz <i>et al.</i> , in press; O. Iribarne, pers. comm.
EASTERN ATLANTIC				
<i>C. furtiva</i> (Loven)	19	muddy, gravelly sand	inshore/offshore (< 200 m)	Tebble, 1966
<i>C. striata</i> (Müller, 1776)	19	muddy sand, gravel, shell	inshore/offshore	Tebble, 1966
<i>C. tigerina</i> (Müller, 1776)	25	sandy mud, gravel, rock	inshore/offshore (to 550 m)	Madson, 1949; Tebble, 1966
<i>C. multistriata</i> (Poli, 1795)	30	?	inshore/offshore (to 2000 m)	Abbott and Dance, 1986
<i>C. tincta</i> (Reeve, 1853)	30	?	?	Abbott and Dance, 1986
<i>C. sulcata</i> (Müller, 1776)	40	?	offshore (to 850 m)	Abbott and Dance, 1986
<i>C. distorta</i> (da Costa)	50	?	inshore/offshore (to > 90 m)	Tebble, 1966
<i>C. flabellum</i> (Gmelin, 1791)	50	?	inshore	Abbott and Dance, 1986
<i>C. septemradiata</i> (Müller, 1776)	51	mud	inshore/offshore (11-183 m)	Allen, 1953; Tebble, 1966
<i>C. nivea</i> (MacGillivray, 1825)	60	?	offshore	Abbott and Dance, 1986
<i>C. varia</i> (Linnaeus, 1758)	64	rocks, muddy gravel, shell	inshore/offshore (to 1000 m)	Allen, 1953; Tebble, 1966
ICELAND				
<i>C. islandica</i> (Müller, 1776)	110	clay, sand, shell	inshore/offshore (to 300 m)	Madson, 1949
BERING SEA				
<i>C. behringiana</i> (Middendorff, 1849)	?	?	offshore (40-150 m)	Bernard, 1983
<i>C. pseudislandica</i> (MacNeil, 1967) ³	75	?	inshore/offshore	MacGinitie, 1959; Bernard, 1979
EASTERN PACIFIC				
<i>C. jordani</i> (Arnold, 1903)	?	?	inshore/offshore (2-60 m)	Bernard, 1983
<i>C. lowei</i> (Hertlein, 1935)	?	?	inshore/offshore (2-175 m)	Bernard, 1983; Keen, 1971
<i>C. amandi</i> (Hertlein, 1935)	40	?	offshore	Abbott and Dance, 1986
<i>C. incantata</i> (Hertlein, 1972)	60	offshore (200 m)	Abbott and Dance, 1986	
<i>C. rubida</i> (Hinds, 1845)	60	rocks, gravel, shell	inshore/offshore (to 183 m)	Rehder, 1981; Kozloff, 1983
<i>C. hastata hastata</i> (Sowerby, 1842)	64	rocks, gravel	inshore/offshore (5-150 m)	Rehder, 1981; Bourne, 1987
<i>C. hastata hericius</i> (Gould, 1850)	83	rocks, sand, mud	inshore/offshore (to 152 m)	Rehder, 1981
SOUTHWEST PACIFIC				
<i>C. dichroa</i> (Suter, 1909)	42	?	offshore (to 100 m)	Abbott and Dance, 1986; Powell, 1979
<i>C. zelandiae</i> (Gray, 1843)	30	undersides of rocks	inshore (to 30 m)	Abbott and Dance, 1986
<i>C. gemmulata</i> (Reeve, 1853)	30	?	inshore (to 30 m)	Abbott and Dance, 1986
<i>C. kiwaensis</i> (Powell, 1933)	33	?	inshore?/offshore	Powell, 1979
<i>C. zeelandona</i> (Hertlein, 1931)	35?	?	inshore	Powell, 1979
<i>C. atkinsi</i> (Pettersd, 1886)	38	?	inshore/offshore?	MacPherson and Gabriel, 1962
<i>C. luculenta</i> (Reeve, 1853)	40	?	offshore	Abbott and Dance, 1986
<i>C. lentiginosa</i> (Reeve, 1853)	40	coral reefs	inshore	Abbott and Dance, 1986
<i>C. taiaroa</i> (Powell, 1952)	43	?	inshore?/offshore	Powell, 1979
<i>C. funebris</i> (Reeve, 1853)	50	?	inshore?	Abbott and Dance, 1986
<i>C. australis</i> (Sowerby, 1847)	60	?	offshore	Abbott and Dance, 1986

Table 2. (continued)

	Shell Height (mm)	Habitat Substratum	Depth ²	Source
<i>C. scabricostata</i> (Sowerby, 1915)	60	?	offshore	Abbott and Dance, 1986
<i>C. squamosa</i> (Gmelin, 1791)	60	?	inshore	Abbott and Dance, 1986
<i>C. dieffenbachii</i> (Reeve, 1853)	64	?	inshore/offshore (to 35 m)	Abbott and Dance, 1986; Powell, 1979
<i>C. delicatula</i> (Hutton, 1873)	70	gravel and shell	offshore (to 200 m)	Bull, in press
<i>C. asperimus</i> (Lamarck, 1819)	100	muddy sand to sand ⁴	inshore/offshore (to 100 m)	Young and Martin, 1989; R. McLoughlin, pers. comm.
<i>C. bifrons</i> (Lamarck, 1819)	150	sandy and coarse bottoms	inshore/offshore (to 100 m)	Young and Martin, 1989; R. McLoughlin, pers. comm.
<i>C. consociata</i> (E. A. Smith, 1915)	?	?	inshore/offshore (to 182 m)	Powell, 1979
NORTHWEST PACIFIC				
<i>C. albida</i> (Arnold, 1906)	?	?	offshore (to 200 m)	Bernard, 1983
<i>C. princessae</i> (Kuroda and Habe)	23?	sand, shell	offshore (to 200 m)	Kuroda <i>et al.</i> , 1971
<i>C. asperulata</i> (Adams and Reeve, 1850)	25	?	inshore (to 20 m)	Abbott and Dance, 1986
<i>C. albolineata</i> (Sowerby, 1887)	25	?	inshore	Abbott and Dance, 1986
<i>C. empresae</i> (Kuroda and Habe)	30?	sand, shell	offshore (to 200 m)	Kuroda <i>et al.</i> , 1971
<i>C. irregularis</i> (Sowerby, 1842)	40	rocks, gravel	inshore/offshore (to 600 m)	Abbott and Dance, 1986; Kuroda <i>et al.</i> , 1971
<i>C. jousseaumei</i> (Bavay, 1904)	40	fine sand	inshore/offshore	Abbott and Dance, 1986; Kuroda <i>et al.</i> , 1971
<i>C. larvata</i> (Reeve, 1853)	40	?	offshore	Abbott and Dance, 1986
<i>C. farreri nipponensis</i> (Kuroda)	?	rock, gravel	inshore/offshore (to 60 m)	Kuroda <i>et al.</i> , 1971
<i>C. lemniscata</i> (Reeve, 1853)	50	sand, shell	inshore/offshore (to 300 m)	Abbott and Dance, 1986; Kuroda <i>et al.</i> , 1971
<i>C. squamata</i> (Gmelin, 1791)	75	rock, gravel	inshore/offshore (to 50 m)	Abbott and Dance, 1986; Kuroda <i>et al.</i> , 1971
<i>C. gloriosa</i> (Reeve, 1852)	75	?	offshore	Abbott and Dance, 1986
<i>C. rosealbus</i> (Scarlatto)	90	silty-sand with pebbles, rocks (rarely sand, shells)	inshore/offshore (13-2030 m)	Silina and Pozdnyakova, 1990
<i>C. nobilis</i> (Reeve, 1852)	118?	rocks	inshore (to 20 m)	Kuroda <i>et al.</i> , 1971
INDIAN OCEAN				
<i>C. ruschenbergerii</i> (Tyron, 1869)	75	?	offshore	Abbott and Dance, 1986
<i>C. senatoria</i> (Gmelin, 1791)	75	?	offshore	Abbott and Dance, 1986
<i>C. townsendi</i> (Sowerby, 1895)	150	?	inshore (to 20 m)	Abbott and Dance, 1986

¹This is not a complete taxonomic listing. Bernard (1983) considers *Hinnites* to be a subgenus, however, because *Hinnites* spp. attach to the substrate by cementation rather than by a byssus this group has been excluded from analyses. In certain instances a species may occupy two geographic regions, however, in order to simplify the table the species is recorded for only one region. An exception was made in the case of *C. islandica*.

²Those species with maximum depth distributions of 30 m are considered inshore species. Note that this division is arbitrary and that not all deepwater occurrences are necessarily offshore.

³There is some debate over whether or not living *C. islandica* occurs in the eastern Pacific although it is reported to occur in the Arctic (Bernard, 1979; Lubinsky, 1980). Bernard (1979) considers *C. islandica* recorded from Point Barrow, Alaska (MacGinitie, 1959) to be *C. pseudislandica* while the more southerly occurring specimens from this collection he considers to be *C. rubida*.

⁴Although widespread on a variety of soft substrata, both juveniles and adults usually found attached by byssus to available solid objects, i.e. rocks, other bivalves, pier pilings (Young and Martin, 1989).

During the Paleozoic, bivalves were primarily restricted to nearshore habitats and it was largely after the Paleozoic that the bivalvia spread offshore to attain their present distributions, replacing the previously dominant articulate brachiopods (Stanley, 1972). From a survey of the habitats occupied by extant species within the genus *Chlamys*, it is seen that there is a high proportion (73%) of species with an offshore distribution, or at least ranging from shallow water to offshore depths (Table 2).

While there is limited ecological information on many of these species, particularly with respect to substrata associations, it would appear that members of the genus have radiated into a variety of habitat types. Substrata associations range from the undersides of shallow water boulders and coral reefs (e.g. subtropical and tropical species) to deepwater muds (e.g. *Chlamys septemradiata* Müller, 1776). Kauffman (1969) classifies byssate species of *Chlamys* as byssate fissure-dwellers. The typical habitats of this group are the under-

sides of rocks, crevices and fissures, reef tunnels, spaces inside root bundles of aquatic plants and similar niches with good water circulation, weak light and good protection from strong wave or current action. The occurrence of *C. septemradiata* on deep-water, flocculent muds (Allen, 1953) represents a unique substratum association within the genus. In fact, in this habitat, *C. septemradiata* serves as a stable settling surface for other sessile invertebrates which otherwise would be subjected to siltation.

Examining species-specific maximum sizes within the genus, it is seen that while most shallow water crevice species are small (< 60 mm), there are several very small ($\leq$ 30 mm) species which are found distributed offshore to great depths (Table 2). The Iceland scallop is one of the largest species within the genus *Chlamys*, attaining a maximum size of about 100 mm in the Newfoundland regions although on the northeastern Grand Bank most scallops are between 60 and 80 mm in shell height (Naidu and Cahill, 1989).

The observed life habit orientation of *Chlamys islandica* on the northeastern Grand Banks is a fully exposed position. Because of the nature of the substratum (dense gravel, cobble), in most instances adult Iceland scallops must assume an epifaunal position on the exposed, upper surfaces of rocks. Only in the case of irregular occurrences of boulders would Iceland scallops be afforded the opportunity to assume a cryptic habit by attaching to the undersides. However, *C. islandica* could be compensated for this apparent lack of refuge through heavy biofouling by barnacles and soft corals in particular, which is often observed on the external surfaces of the upper valve (KDG, pers. obs.). While this may not decrease the risk of predation from chemosensory orienting predators (see Lake *et al.*, 1987), presumably it would be an advantage in the case of visually cueing predators, in particular, fish. Epizoid associations have been studied for several species. Epizoid sponge cover of the valves of *C. varia* (Linnaeus, 1758) and *C. asperrima* (Lamarck, 1819) is known to provide protection from predatory starfish (Forester, 1979; Chernoff, 1987; Pitcher and Butler, 1987). *Chlamys dieffenbachii* (Reeve, 1853) is almost invariably enveloped in living sponge (Powell, 1979) while *C. hastata* and *C. rubida* (Hinds, 1845) are regularly colonized by sponges that form thick coatings (Kozloff, 1983).

In summary, from the results of this study, and the observations of others, it would appear that *Chlamys islandica* is restricted to habitats with coarse substrata although this includes a range of sediment types from gravelly sand to gravel, cobble and shell mixtures. This would appear to be due to a requirement for byssus attachment at all life history stages. Within the genus, other species known to be byssally attached to substrates as adults include: *C. asperrima* (Young and Martin, 1989), *C. varia* (Rodhouse and Burnell, 1979), *C. irregularis* Sowerby, 1842, *C. squamata* Gmelin, 1791, *C. farreri* Jones and Preston, 1904 and *C. nobilis* Reeve, 1853

(Kuroda *et al.*, 1971). Frequency of byssal attachment to the substratum decreases with age in *C. tehuelcha* although the capacity to form a byssus is not lost in the largest individuals (Orensanz *et al.*, in press). While occupation of habitats with coarse substrata appears to be typical of members of the genus, there is at least one example (*C. septemradiata*) of radiation into a deepwater habitat characterized by flocculent muds, presumably with a consequential loss of byssus attachment.

ACKNOWLEDGMENTS

We thank the Centre for Cold Ocean Research and Engineering (Memorial University of Newfoundland), the Atlantic Geosciences Centre (Bedford Institute of Oceanography, Dartmouth, Nova Scotia), the Offshore Geotechnics Program of the Federal Panel on Energy Research and Development, T. Folkes, M. Lewis and the Captain and crew of the C.S.S. HUDSON for logistic support. We thank J. A. Hutchings and S. Naidu for reviewing the manuscript, G. Carmichael for graphics, and D. Pitcher for assistance with data analyses. We are grateful to S. Shumway, M. Bricelj, O. Iribarne, P. Young and R. McLoughlin for providing unpublished data or information sources for Table 2.

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Date of manuscript acceptance: 1 October 1990

Anatomical and behavioural studies on vision in *Nautilus* and *Octopus*

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Abstract. *Nautilus* is a cephalopod that is primitive in many respects, and is often considered to be a "living fossil". The eye of *Nautilus* is apparently a primitive feature, acting as a pin-hole camera and lacking any lens or other dioptric apparatus. In contrast, in *Octopus* and most other coleoid cephalopods, there is a well formed spherical lens. The basic structure of the retina is similar in the two animals, but there are also a number of important differences: the microvilli of the receptors of *Nautilus* do not form a regular rectilinear array as they do in *Octopus*; the microvilli from neighbouring receptors overlap, which does not occur in *Octopus*; the supporting cells have a different structure; the nuclei of the supporting cells and receptor cells are distributed either side of the basement membrane in *Octopus*, but not in *Nautilus*; cilia are present in the retina of *Nautilus* but not *Octopus*; and the myeloid bodies are much more developed in *Nautilus*.

Both behavioural experiments and calculation show that, as expected on anatomical grounds, visual acuity and sensitivity are much better in *Octopus* than *Nautilus*. Reasons for the limitations in the visual capabilities of the two animals are discussed.

Nautilus is the last surviving genus of a group that arose in the Triassic, and has apparently changed little since Cretaceous times, at least as far as we can judge from the shell. The animal shows many apparently primitive features, such as the lack of an ink sac or chromatophores, the external shell, the funnel formed of two overlapping lobes, and the simple pin-hole camera eye lacking any lens or other dioptric apparatus (e.g. Morton, 1967). The ancestry of the genus has been discussed by, among others, Teichert and Matsumoto (1987), who concluded that it can truly be called a "living fossil".

The first octopods, on the other hand, are found in the Upper Cretaceous (Donovan, 1977), and the group must be counted among the most developed invertebrates in existence. The octopus eye, which resembles that of most other coleoid cephalopods, has in contrast to *Nautilus* a very well-developed lens and superficially looks remarkably similar to the eyes of vertebrates. The present paper compares the structure and function of the eyes of these two very different cephalopods.

STRUCTURE OF THE EYE IN *NAUTILUS* AND *OCTOPUS*

The most obvious difference between the eyes of *Nautilus* and *Octopus* is the complete lack of any dioptric apparatus in the former genus. The pupil in *Nautilus* opens directly to the sea, and the eye must act as a pin-hole camera. The eyes of octopuses in contrast have well developed spherical lenses, which, as in most fishes, have focal lengths

about 2.5 times their radius (Matthiessen's ratio) and are well corrected for spherical aberration (Sivak, 1982; Sroczyński and Muntz, 1985). The overall size of the eyes of the two animals is, however, similar, and both animals have contractile pupils that are elongated in the horizontal direction (Muntz, 1977; Hurley *et al.*, 1978). Figure 1 shows the general appearance of the eyes of *Nautilus pompilius* Linnaeus and *Octopus vulgaris* Lamarck.

Descriptions of the retinal anatomy of *Nautilus* can be found in Barber and Wright (1969), Muntz and Raj (1984), and Muntz and Wentworth (1987); and of *Octopus* in Young (1962a, 1971) and Yamamoto *et al.* (1965). These papers also give references to earlier work. Following convention, in this paper the segments of the receptors facing the light, which contain the photopigment, will be referred to as the distal or outer segments, and the nuclear region as the proximal or inner segment.

The basic elements of the retina in both *Octopus* and *Nautilus* are the receptor cells, with distal segments consisting of a central core from which the microvilli (which contain the visual pigment) radiate outwards, and the supporting cells with their processes lying between the receptor cell outer segments (Figs. 2, 3). The packing of the receptor cells is roughly similar in the two species. Thus, there are about 20,000 receptor cells mm⁻² in *N. pompilius*, varying little over the retina (Muntz and Raj, 1984), and between 18,000 and 55,000 mm⁻² in *O. vulgaris*, depending on retinal position (Young, 1971). Although basically similar, there are however also a number of important differences between the two species, which can be summarised as follows.

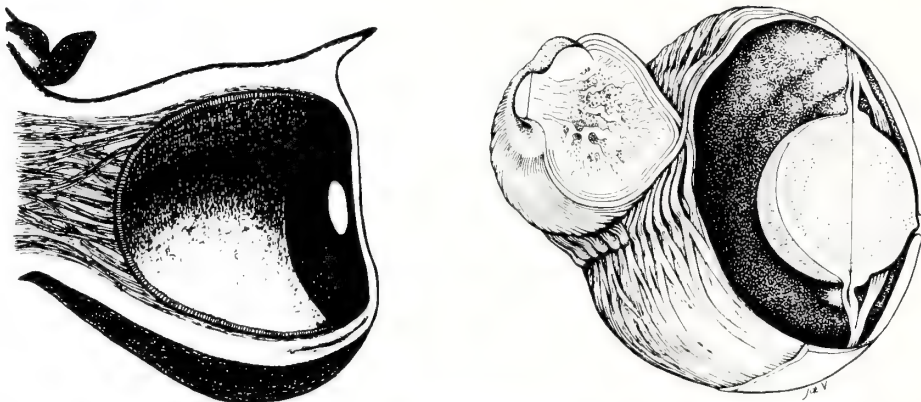


Fig. 1. Left, vertical section through the eye of *Nautilus* (from Willey, 1902). Right, vertical section through the eye and optic lobe of *Octopus* (from Young, 1962b).

(i) Transverse sections through the outer segments of the retinal receptors of *Nautilus* show that there are usually five or six (occasionally four or seven) bundles of microvilli running out from each receptor body to the bodies of neighbouring receptors. The receptors thus form a roughly hexagonal array (Fig. 2a). The microvilli from neighbouring receptors within a given bundle often interdigitate, although the extent of this interdigitation is not clear. The processes of the supporting cells run out in groups between these bundles of microvilli.

In contrast in *Octopus* the receptor outer segments form a rectilinear array, with the microvilli oriented vertically or horizontally with respect to gravity (Fig. 3a). The microvilli of each receptor remain strictly segregated from those of the neighbouring receptors, with no interdigitation.

(ii) The structure of the supporting cells is quite different in the two animals. In *Nautilus* each cell has a number of fine microvillous processes which project out between the receptor outer segments in groups, whereas in *Octopus* each supporting cell has a single process, which is much larger and contains screening pigment. In the former animal the cell nuclei of both the receptors and the supporting cells lie distal to the basement membrane, whereas in *Octopus* the supporting cell nuclei lie distal and the receptor cell nuclei proximal to the basement membrane.

(iii) In *Nautilus* the supporting cells have cilia, as well as the microvillous processes that extend between the receptors. It is not certain whether the receptor cells have cilia as well. Ciliary structures have not been reported in the retina of any other adult cephalopod, although the photosensitive organs of many animals have receptors of ciliary origin, or cilia, presumed not to be photosensitive, intermingled with the receptors (Vanfleteren, 1982).

(iv) The inner segments of *Nautilus* photoreceptors have complex myeloid bodies, which often have the appearance of a tubular structure, or a series of wavy plates (Fig. 2). It has been argued that this apparently complex structure consists of a series of dimpled plates, stacked in register

above each other (Muntz and Wentworth, 1987). In *Octopus* and the other coleoid cephalopods, the myeloid bodies are reduced to a few membranous strands.

It is interesting that some of the characteristics by which the *Nautilus* retina differs from that of adult octopuses also have been found during the development of the embryos of coleoid cephalopods. Thus in the cuttlefish *Sepiella japonica* Sasaki, embryos have cilia on both receptor and supporting cells, the nuclei of both the receptor cells and the supporting cells lie distal to the basement membrane, and the supporting cells send long microvillous processes out among the whole length of the receptor outer segments (Yamamoto, 1985). Work in progress shows a similar situation in the embryos of the Australian octopuses *Octopus pallidus* Hoyle and *O. australis* Hoyle (Wentworth and Muntz, unpub. data).

BEHAVIOURAL STUDIES

To date, no studies of vision have been carried out with *Nautilus* using any form of learnt behaviour, and it is not known how far the animals are capable of learning. However, *Nautilus* shows two well developed forms of innate visual behaviour, the positive phototactic response and the optomotor response, which have been used to determine the animals' visual acuity, and also their absolute and spectral sensitivities (Muntz and Raj, 1984; Muntz, 1986, 1987).

As we should expect for an animal with an eye having the simple optics of a pin-hole camera, visual performance in *Nautilus* is very poor compared to that of animals with lens bearing camera eyes. The minimum separable visual acuity, for example, measured using the optomotor response, lies between 5.5° and 11.25°, which agrees well with values calculated on the basis of the gross dimensions of the eye and pupil, and with expectations based on photographing a visual test chart using a scale model of the eye (Muntz and Raj, 1984). This can be compared with values of about 5°

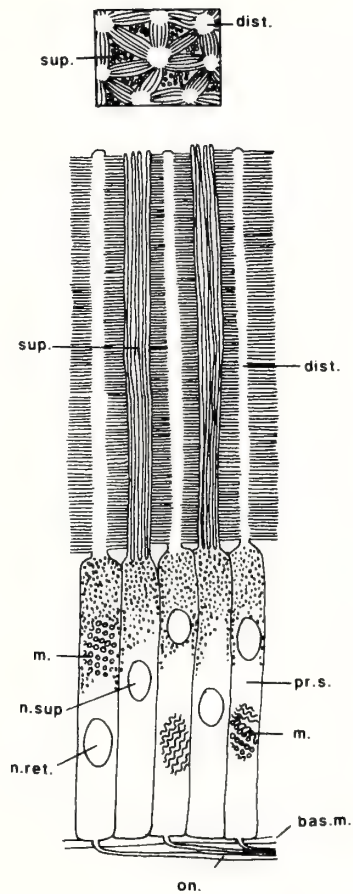


Fig. 2. Diagram of the structure of the retina of *Nautilus*, as seen in tangential (above) and radial section (below). The diagram is not to scale: in particular the horizontal dimensions have been exaggerated compared to the vertical dimensions for clarity. The mean length of the distal segments is in fact about 360 μm , and of the proximal segments 100 μm , and the mean centre to centre distance between adjacent receptors 3.5 μm : there is little variation over the retina (bas.m., basement membrane; dist., receptor distal segment with microvilli radiating from a central core; pr.s., receptor proximal segment; sup., processes of supporting cells; m., myeloid body; on., optic nerves; n.sup., nucleus of supporting cells; n.ret., nucleus of retinal cell).

obtained with octopuses, various fishes and two aquatic mammals (Table 1): even with the most favourable estimate of 5.5⁰ the performance level of *Nautilus* is over 60 times worse than for these other aquatic animals.

By using the positive phototactic behaviour of *Nautilus* it has also been possible to determine its absolute sensitivity to tungsten light (Muntz, 1987). In itself this result is not particularly useful, because the spectral output of the tungsten source used was very different from that of the light to which the animal will be exposed in its natural environment. However, given a knowledge of the spectral transmission of the water in which the animals live, the spectral quality of the daylight reaching the surface of the sea, and the animals' own spectral sensitivity, it is possible from these results to

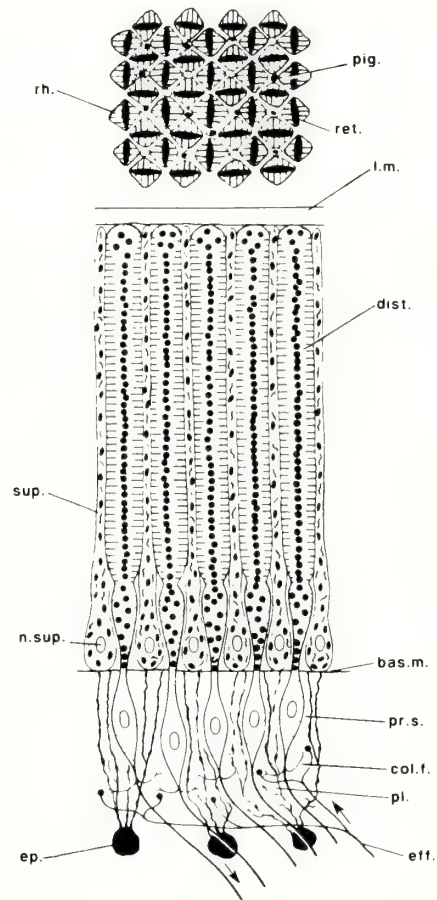


Fig. 3. Diagram of the structure of the retina of *Octopus*, as seen in tangential (above) and radial section (below), from Young (1962a). Not to scale: in fact the distal segments vary between about 60 μm and 180 μm in length depending on retinal position, while the proximal segments are about 90 μm long and the individual rhabdomeres about 5 μm in width (Young, 1962a) (rh., rhabdome; pig., pigment granule at centre of rhabdome; ret., retinal cell; l.m., limiting membrane; dist., distal segment of receptor; bas.m., basal membrane; pr.s., proximal segments of retinal cells; col.f., fine dendritic collateral of retina cell; pl., retinal nerve plexus; eff., ending of efferent fibre in retina; ep., epithelial cell; n.sup., nuclei of supporting cells; sup., processes of supporting cells).

calculate the maximum depth at which surface light would be visible at all to *Nautilus*. Reasonable estimates for the first two factors are available, and the animals' spectral sensitivity was taken to be the same as the absorption spectrum of its visual pigment (see Muntz, 1987 for details). It appears that some daylight should be visible to *Nautilus* down to 800 m, which is slightly deeper than the maximum depth at which the animal is found. This is, however, considerably less than the maximum depth at which daylight should be visible to deep sea fishes, which has been calculated by Clarke and Denton (1962) as over 1000 m. Calculations based on the dimensions of the *Nautilus* eye also indicate that *Nautilus* will be less sensitive, by about 2 log units, than a fish or cephalopod that has a camera eye with a lens obeying

Matthiessen's ratio (Muntz and Raj, 1984).

Finally, the positive phototactic behaviour has also been used to determine spectral sensitivity directly (Muntz, 1986). The sensitivity curve obtained agreed well with the absorption spectrum of the extractable visual pigment, which was itself well fitted by Dartnall's (1953) visual pigment nomogram for an A_1 -based pigment with its maximum at 467 nm.

In contrast to *Nautilus*, octopuses learn visual discriminations very readily, and a great deal of information is now available on their visual capabilities (Wells, 1978; Messenger, 1981 for reviews). Most of this work has concerned higher visual functions, such as the ability to discriminate shapes, the mechanisms by which such discriminations are learnt, and the function of the various parts of the central nervous system. Comparatively little work has been done on the animals' more basic visual capabilities, such as sensitivity or visual acuity, which are probably more directly related to the optics of the eye and the structure and function of the retina, and which can be compared to the data that are available for *Nautilus*. In the case of sensitivity, for example, there appear to have been no studies at all carried out on coleoid cephalopods using learning, and only one study involving innate behaviour, in which the spectral sensitivity of *Loligo pealei* Lesueur larvae was measured using the positive phototactic response in a manner rather similar to that used with *Nautilus*. The results were also similar show-

ing a smooth bell-shaped spectral sensitivity curve maximal at around 480 nm and compatible with a single visual pigment (White, 1924).

A few behavioural studies have been carried out on the visual acuity of octopuses. Thus Sutherland (1963), using a training situation, obtained an estimate of 17' for *Octopus vulgaris*, and Packard (1969), using the same species and the optomotor response, found that in very small specimens (< 3-22g) acuity improved with size. The most recent studies on acuity in octopuses (Muntz and Gwyther, 1988a, 1989) used fully grown animals and a two choice learning situation, and the stimuli were gratings of equally spaced black and white stripes oriented vertically, horizontally, or obliquely at 45°. The animals were trained to discriminate these gratings from each other or from a uniform gray stimulus, and visual acuity was taken as the separation between the bars of the gratings where performance reached chance levels. The results showed that the minimum separable visual acuity of *O. australis* and *O. pallidus* is about 5' (Fig. 4). With gratings close to the animals' threshold, performance with the vertical gratings was best, and with the horizontal gratings worst, but the effect was not large.

The ability of *Octopus pallidus* and *O. australis* to discriminate distances has also been determined behaviourally, using the animals' tendency to attack the nearer of two stimuli presented simultaneously (Muntz and Gwyther, 1988b). Assuming that the animals are using accommoda-

Table 1. Minimum separable visual acuities, in minutes of arc, of various aquatic animals measured behaviourally using gratings. Learnt discriminations were used in all cases except *Nautilus* where the optomotor response was used.

Species	Acuity	Reference
MAMMALS		
Harbour seal <i>Phoca vitulina</i> Linnaeus	8.3	Schusterman and Balliet, 1970
Stellar Sea Lion <i>Eumetopias jubata</i> (Schreber)	7.1	Schusterman and Balliet, 1970
TELEOST FISHES		
Convict fish <i>Microcanthus strigatus</i> (Cuvier and Valenciennes)	4.9	Yamanouchi, 1956
Minnow <i>Phoxinus laevis</i> Linnaeus	10.8	Brunner, 1934
Skipjack tuna <i>Katsuwomis pelamis</i> Linnaeus	5.5	Nakamura, 1968
Little tuna <i>Euthynnus affinis</i> (Cantor)	7.4	Nakamura, 1968
Cichlid fish <i>Aequidens portalegrensis</i> (Hensel)	5.8	Baerends <i>et al.</i> , 1960
CEPHALOPODS		
<i>Nautilus</i> <i>Nautilus pompilius</i>	330-670	Muntz and Raj, 1984
Octopus <i>Octopus pallidus</i> <i>O. australis</i>	5.0	Muntz and Gwyther, 1988a

tion to estimate distance, which various tests indicated is the most likely mechanism, the animals can detect blurring of points on the retinal image comparable in size to a single retinal receptor, and lens displacements of around 10 μm .

Finally, training experiments have shown that *Octopus vulgaris* can discriminate the plane of polarised light (Moody and Parriss, 1961), and also it has been shown that two species of decapod larvae orient themselves to the plane of polarised light (Jander *et al.*, 1968). It is not known whether *Nautilus* has this ability.

DISCUSSION

The evolution of the camera eye has attracted interest ever since Darwin [1859 (reprinted 1958)] listed it as one of the 'organs of extreme perfection and complication', and wrote that to believe that such organs could have been formed by natural selection seems 'absurd in the highest possible degree'. Darwin's solution to the problem was to suggest that the eye must have arisen through numerous inheritable gradations, each of which was useful to its possessor, and that while strictly we should look for such gradations among the animal's lineal ancestors, we are usually forced to look at living species of the same group to see what gradations are possible.

The eye of *Nautilus* could be taken as such a gradation on the route to the complex eyes of the more recent

cephalopods. Even though *Nautilus* vision is poor, nevertheless its visual behavior is precise, in that in the optomotor response they follow the stripes accurately without visible lag, and in the phototactic situation if the difference between the stimuli is well above threshold the brighter light is chosen on every occasion. The eyes are also stabilised with respect to gravity by means of the statocysts (Hartline *et al.*, 1979). These facts suggest that vision is important to the animal. It is not, however, clear what use they make of such poor vision in their normal life. The habitat of *Nautilus* often has strong currents, and the optomotor behaviour could be related to holding station under these conditions. It could also be that the positive phototactic behaviour is related to bioluminescence, which is a major source of light at depth in the sea. *Nautilus* is often trapped in association with deep water bioluminescent shrimps, which also feed on decaying animal material, and moving towards bioluminescence could help take the animals towards their food. Finally, *Nautilus* shows diurnal vertical migrations (Carlson *et al.*, 1984; Ward *et al.*, 1984), and vision could be a factor in this behaviour. Without further information on the normal behaviour of the animals however, these remain speculations.

In the case of *Octopus* we have no behavioural evidence on its visual sensitivity, although presumably it is considerably better than that of *Nautilus*. The minimum separable visual acuity of 5' for *Octopus* is comparable to that of fishes and aquatic mammals (Table 1). Nevertheless, it is not clear why the acuity is not even better than this. The retinal mosaic is rather finer than necessary for the acuity that is in fact achieved (Muntz and Gwyther, 1988a), and the pupil size is large enough that diffraction will not be limiting. In *Eledone cirrhosa* (Lamarck), another octopod, spherical aberration is far less than would be needed to limit acuity to this level (Sroczyński and Muntz, 1985). Furthermore, terrestrial animals can achieve much better acuities; in the case of humans, for example, the minimum separable lies between 0.5' and 1' (e.g. Senders, 1948), and in the American kestrel, *Falco sparverius* Linne', the acuity, measured behaviourally using square wave gratings, is 0.19' (Fox *et al.*, 1976). Since, however, the minimum separable acuity, measured behaviourally, has been found to be about 5' for all aquatic animals where it has been measured, it could be the environment itself that is limiting. While not very many data are available on the subject, it is clear that high spatial frequencies are particularly heavily attenuated by the water body itself, and it could be that the ability to resolve very fine detail is consequently irrelevant (see Muntz, 1990 for further discussion).

The ability of octopuses and other coleoid cephalopods to discriminate the plane of polarisation of light is usually attributed to the regular rectilinear array of the microvilli of their receptors (Moody and Parriss, 1961). *Nautilus* lacks such a rectilinear array. Nevertheless, the microvilli within any

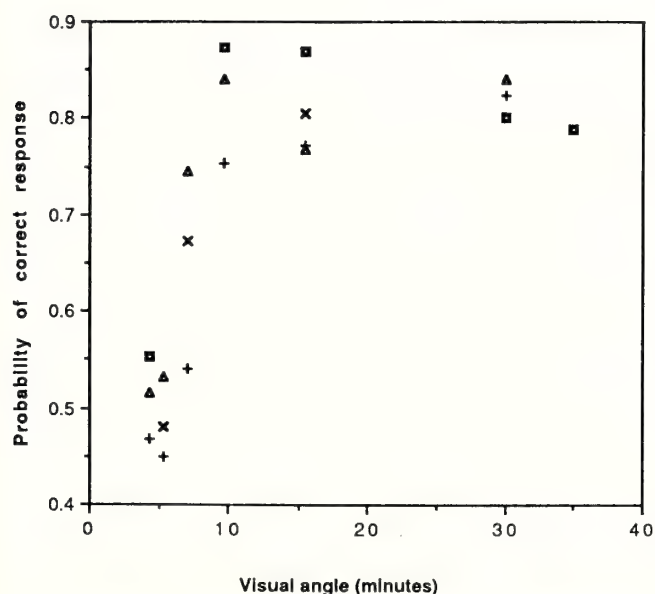


Fig. 4. Visual acuity of *Octopus*, measured behaviourally for various stimulus combinations. The animals weighed between 59 and 1134g. ▲, vertical gratings against grey; +, horizontal gratings against grey; X, oblique gratings against grey; ■, vertical gratings against horizontal gratings. Data from Muntz and Gwyther (1988a, 1989).

given bundle remain parallel to each other, and so plane polarised light should still be able to affect the receptors differentially, even though there would be no precise relationship between the plane of polarisation and the receptors stimulated. It would be interesting to know whether *Nautilus* can show any differential response to polarised light.

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Complex learning in *Octopus bimaculoides*

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Abstract. In order to investigate complex learning in *Octopus bimaculoides* (Pickford and McConnaughey), I presented subjects with a series of combinations of mollusc shells. Combinations consisted of two shells of one type and an odd shell of another type. The shells were suspended in the octopuses' home tanks, and the animals were rewarded with food for correctly grabbing the odd shell. Associative learning was demonstrated by the subjects' eventual mastery (70 - 100% success rates) of each combination in a series (A+ B- B-), (C+ D- D-) ... By mastery of new combinations of the same stimuli, (A+ D- D-), (C+ B- B-) ..., subjects demonstrated transfer of learning. Learning improved across successive combinations, evidence for learning set formation. However, because octopuses did not learn to choose the odd stimulus when trained only with non-repeating combinations, no evidence indicated that the octopuses formed the relative class concept of oddity.

The demonstration of complex learning in cephalopods could provide important insight into the evolution of cognition. Most research on complex learning has focused on higher vertebrates. However, an ecological approach suggests that complex learning could evolve whenever it was adaptively advantageous (Shettleworth, 1984). Several investigations have suggested a connection between complex learning abilities and sociality (see Humphrey, 1976; Essock-Vitale and Seyfarth, 1986). Wells (1978) has argued, however, that because the predominantly solitary octopuses have no obvious means of self defense and live in a highly competitive environment, they also could show complex learning. The experiments reported here test this proposal.

I presented *Octopus bimaculoides* (Pickford and McConnaughey) with three objects, two alike and one different, and rewarded them with small pieces of squid if they grabbed the odd one. Using this methodology, I could pose a series of problems differing in the complexity of learning required for successful mastery.

The first question was, could the octopuses learn to choose a particular shell, in repeated presentations of the same combination? Simple associative learning of this sort has previously been demonstrated with *Octopus vulgaris* (Cuvier) (Wells, 1978).

The second question was, could the octopuses still pick the correct shell if known shells were arranged into new combinations? Positive results would show transfer of learned response tendencies.

Thirdly, as the animals gained experience in learning shell combinations, would they improve at learning new but similar tasks? An ability for learning to learn, or developing a learning set, has not been shown previously in an invertebrate, although the related task of learning reversals has

(Mackintosh, 1965; Morrow and Smithson, 1969).

Lastly, could the octopuses eventually generalize and immediately choose the odd shell when presented with new combinations? Generalization has been shown in tactile discriminations for *Octopus vulgaris* (Wells and Young, 1970). This task is particularly significant because oddity is an abstract concept, defined only in relationship to other objects and not by any attribute of the object itself. So far, only higher invertebrates have shown evidence of forming such relative class concepts (Thomas, 1980; Lombardi *et al.*, 1984; Thomas and Noble, 1988).

METHODS

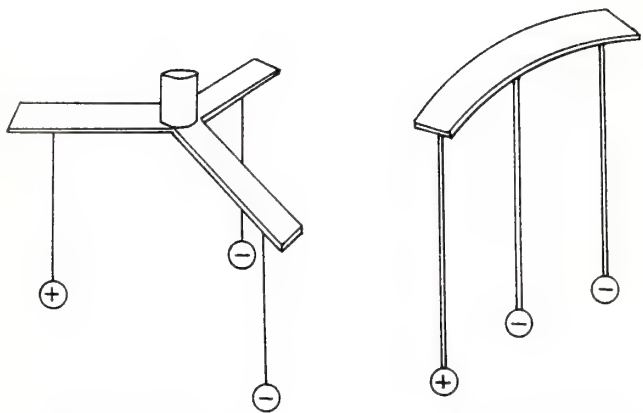
EXPERIMENT I

Subjects were three wild caught adults of undetermined sex which had been living in the laboratory a full six months before experiments began. In the laboratory, they were nocturnal and not easy to clock-shift. Therefore, experiments were performed at night under red light.

The octopuses were trained initially to take small pieces of frozen squid from a rod and, later, to grab a single plastic triangle or square on the end of a rod to get a food reward. On days when they were unsuccessful with discrimination tasks, I fed them after trials using the rod alone, up to their minimum daily intake.

Trials consisted of presenting three stimuli, two alike and one different. Six different combinations were used (Appendix 1). The combinations presented were composed of mollusc shells varying in color, texture, and shape, except for combinations four and five, which consisted of plastic shapes which varied in both texture and shape.

I suspended the stimuli on nylon monofilament in the



Experiment I

Experiment II

Fig. 1. Apparatus for presenting combinations of shells. Location of the odd, positive stimulus (+) was determined randomly. In Experiment I, the shells were suspended on monofilament; in Experiment II, they were attached to acrylic rods.

octopuses' tanks (Fig. 1). The location of the odd object was determined randomly, with the constraint that in half of the presentations it was in the front half of the tank, and in half it was in the back. A subject was then given two minutes in which to grab one of the stimuli. Responses were usually immediate. A correct response was promptly rewarded with a small piece of squid. I gave each subject eight trials per learning session, ten to fifteen minutes apart, with two learning sessions per day, 11 sessions per week.

In Experiment I, each of the six combinations was presented for 11 sessions or until all subjects reached a success rate of greater than 50% for three successive sessions. The second combination was cut short because one of the shells was shattered by a particularly vigorous grab. Octopuses were then retested for two or three sessions with each familiar combination. They were then each given an equal number of presentations with three of eight arbitrarily chosen new combinations of the same, familiar stimuli. Positive, rewarded shells remained positive and negative, unrewarded shells remained negative; however, the particular combinations of positive and negative stimuli were new.

Experiment I ran for a total of 119 sessions across 12 weeks. Response rates averaged 43% for the three octopuses. For each octopus, sessions with fewer than two grabs were eliminated from the study.

EXPERIMENT II

Subjects were three freshly caught *Octopus bimaculoides*, just reaching sexual maturity, one female and two males. Initial training was carried out as described above. Trials consisted of presentation of three stimuli, two alike and one different, as before. All stimuli used were mollusc shells (Appendix 2). Shells were presented in a line (Fig. 1) to correct for the location bias found in Experiment I. I gave the

octopuses ten trials each learning session, spaced five to ten minutes apart, with one session per day, six days per week.

In this experiment, every combination presented to a subject (each trial) was novel. Sixty pairs of shells were used, with every type of shell presented both as a positively rewarded odd shell and as a negative pair, in order to control for the possibility that subjects had attended to some attribute other than oddity. Each type of shell was therefore seen by the octopuses only twice within each week: once as a positive single shell, and once as a negative pair of shells. The only way to solve this problem successfully would be to employ the relative class concept of oddity.

Combinations of shells were determined randomly with the constraint that positive odd shells differed from the negative pair by two of the three features of color, texture, or type (bivalve or gastropod) (Appendix 2). Orders of presentation and locations of the odd shells were randomized.

Simple reinforcement trials were given at the beginning and end of each training session and randomly interspersed among the oddity discrimination problems. These trials consisted of presenting the octopus with the single plastic triangle or square used in initial training. Octopuses were rewarded for grabbing the shape (no choice or discrimination was involved). Response rates in discrimination tasks were 89%. To reduce any inadvertent cuing, beginning with the second week of trials a gauze curtain was draped between the experimenter and the octopuses, with red lights only on the octopuses' side.

RESULTS

EXPERIMENT I

Performances of the three octopuses were statistically indistinguishable (contingency table for three subjects versus correct or incorrect response: $\chi^2 = 0.694$, d.f. = 2, $P > 0.70$). Results were therefore pooled.

Octopuses showed a strong bias towards objects in the front of the tank in the first experiment ($\chi^2 = 107.15$, d.f. = 1, $P < 0.01$); subjects grabbed front stimuli more frequently and less accurately. Response rates were low and not related to success rates (Figs. 2, 3).

Could the octopuses learn to choose correctly one object out of a combination with repeated presentations? Success rates for the first combination, in terms of correct choices as a percentage of total grabs (Fig. 2), showed clearly that the combination was learned and retained. Despite the small sample size and wide variability in performances, learning curves for the first 11 sessions of all six combinations (Fig. 3) demonstrated that the octopuses were able to learn to choose the correct object consistently.

Could they still choose the correct shell if known shells were recombined in new ways? This task required the ability

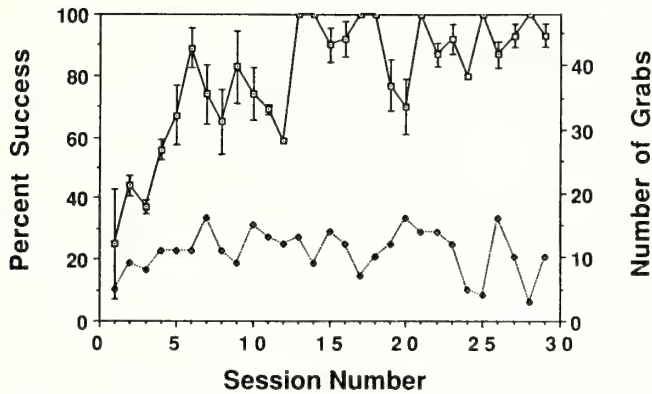


Fig. 2. Mean success rates (squares) (number of correct grabs as percentage of total grabs) with standard errors and total number of grabs (diamonds) across sessions, for the three octopuses on the first discrimination combination.

to remember five (combination two had been broken) simultaneous discriminations, or positive or negative attributes of at least five of the ten different stimuli. Their high success rate with the familiar arrangements shows good retention over this time period, and also shows that five simultaneous discriminations can be mastered by these octopuses. That they performed equally well on the original and new combinations ($t = 1.01$, d.f. = 26, $P > 0.20$) is evidence of transfer of learning (Fig. 4); they could use the learned information about member shells of a combination in a new context.

Could the octopuses improve at the task of learning new combinations? Variability was too high and sample sizes too small for comparisons to be made among slopes and intercepts of the six learning curves in the series. However, performances on days one and five (Fig. 5) (or for combination two, the last two completed learning sessions) across the six sequential combinations showed a trend toward improvement in first-day performances. For the sixth combination, a second observer was present on the first day and not on the fifth, a difference that could have affected performances. A paired comparisons test on all six combinations for the two days showed significant effects for both day and combination (days, $F = 18.98$, d.f. = 1,5, $P < 0.01$; combinations, $F = 10.81$, d.f. = 5,5, $P < 0.025$). The octopuses thus both learned the individual combinations and improved across the series. Sample sizes were too small to control for any effects of shells (combinations 1-3, and 6) versus plastic objects (combinations 4-5).

The last question was, could the octopuses generalize and choose the odd object after learning a number of different combinations? Their very high success rates on day one for the later combinations suggested that they might have formed the relative class concept of oddity. Experiment II was undertaken to explore this possibility further.

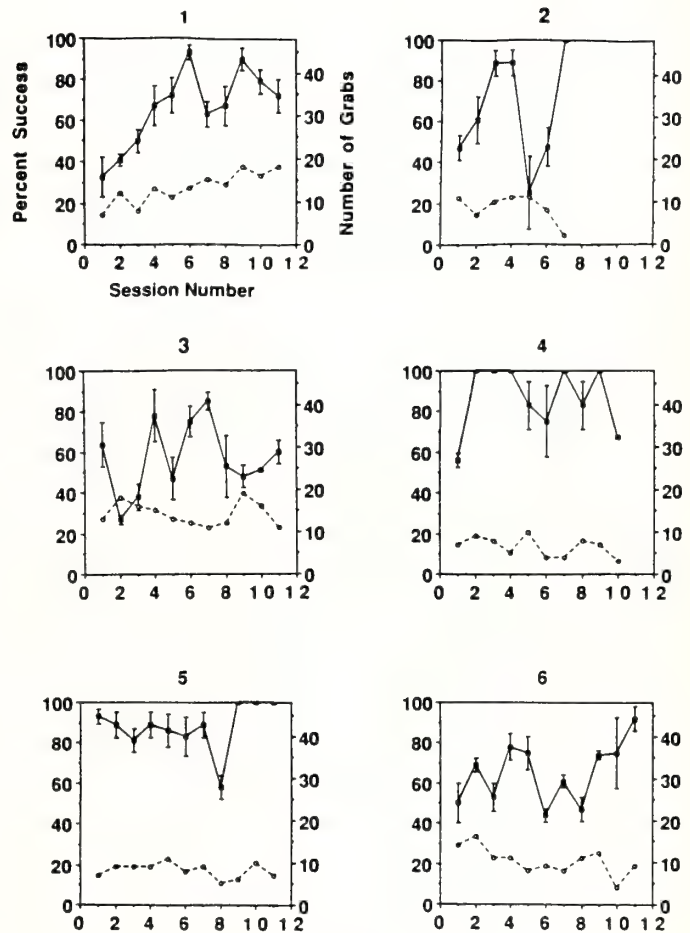


Fig. 3. Mean success rates and standard errors for each of the six combinations ($n=3$). Dotted lines indicate the total number of responses. Note the above-random performance on all combinations, despite wide variation.

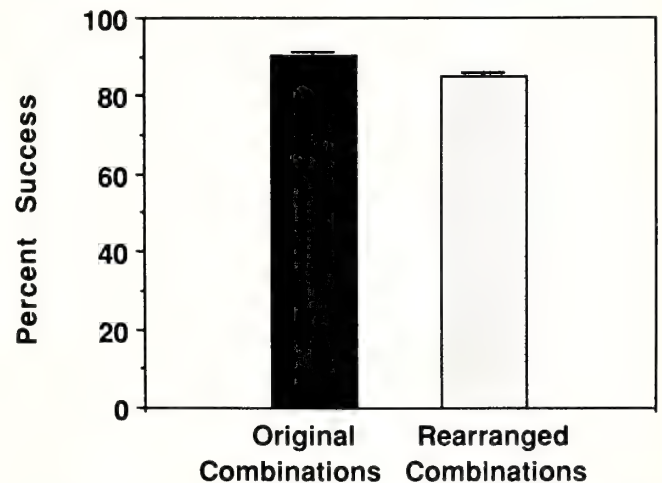


Fig. 4. Mean success rates and standard errors for the three octopuses with five original combinations ($n=13$) and with eight new combinations of familiar stimuli ($n=15$).

EXPERIMENT II

Performances of the three octopuses were once again statistically indistinguishable (contingency table for three subjects versus correct or incorrect response: $\chi^2 = 0.132$, d.f. = 2, $P > 0.90$). Results were therefore pooled.

The performance of the octopuses in choosing the odd object showed no clear improvement over time (Fig. 6). When mean success rates are separated by week (or by natural breaks), performances appeared slightly, but not significantly, better than completely random (33%). The means by week were 38, 36, 36, 47, and 40% ($n=18$).

The periodicity found in this learning curve (Fig. 6) was unexpected. It did not correspond to the six session work weeks and was not evident in all three individuals' performances (Fig. 7).

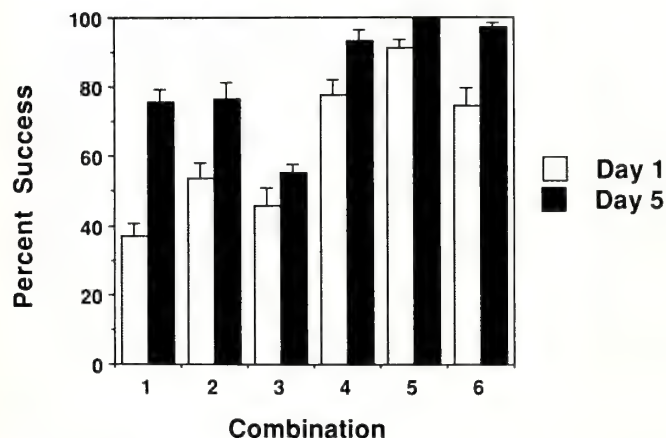


Fig. 5. Mean success rates and standard errors for days one and five for each of the six sequential combinations.

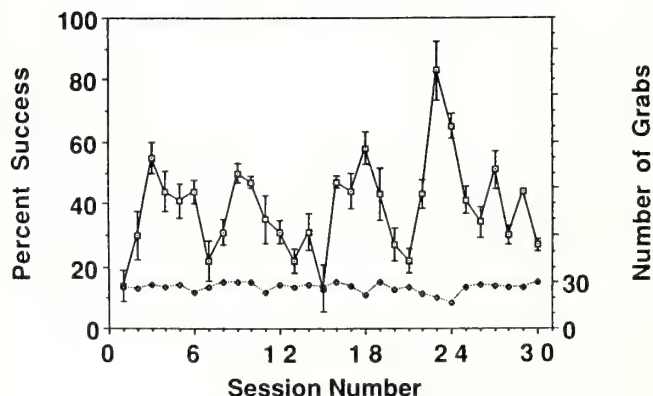


Fig. 6. Mean success rates (squares) with standard errors ($n=3$) and total numbers of responses (diamonds) across sessions in Experiment II when every combination presented was novel.

DISCUSSION

Octopus bimaculoides, a small octopus from the southern California coast, is a solitary, nocturnal predator, feeding primarily on gastropods but also on bivalves, polychaete worms, fishes, and crabs (Forsythe *et al.*, 1984). It lives in dens or burrows and interacts rarely with other octopuses (Lang, 1990). Generations do not overlap, which precludes the level of social learning available to even the most solitary of mammals. Laboratory-reared octopuses have the reputation of being slower to learn than those caught wild, perhaps an indication that learning takes place normally in their environment.

The particular tasks in these experiments do not relate directly to any activities known to be performed in the wild. However, they are comparable to experimental tests used to assess cognitive abilities in a wide range of species (Thomas, 1980).

In Experiment I, the octopuses' mastery of the simple task of learning to choose a particular shell after repeated presentations of the same combination is clear evidence of associative learning. This result is consistent with findings for *Octopus vulgaris* (Wells, 1978) as well as other invertebrates (Corning *et al.*, 1976).

Transfer of learning was also evident from performances on new combinations of familiar stimuli, an indication that their learning was not tightly dependent on context. Whether they based their choices on learned positive identities, learned negative identities, or both, cannot be determined from this experiment. However, response patterns suggest that they did in fact learn both. The octopuses normally sat in one of the top front corners of the tanks during trials. During the first experiment, they quickly stopped swimming to the far side of the tank (approximately an extra 20 cm) in order to grab the far stimulus. Therefore, they had to decide not only which stimulus to grab, but which stimuli not to grab, since the two nearby shells might both be negatives. They were highly successful at learning these discriminations, an indication that they had, in fact, learned both positive and negative shell identities.

That learning was retained over time was shown in the transfer of learning trials. The octopuses' success rate with the first combination was 80%, when retested after five weeks of experience with other combinations. Informal observations suggest that these animals might show retention even for months.

Learning set formation, or learning to learn, implies that something beyond recognition of objects has been learned. It could be only to attend carefully to relevant stimuli. Or, it could involve remembering previous choices and outcomes so as to arrive more quickly at correct solutions. Although learning set formation has not been shown previously in invertebrates, the related task of learning reversals has been mastered by isopods (Morrow and Smithson, 1969) and

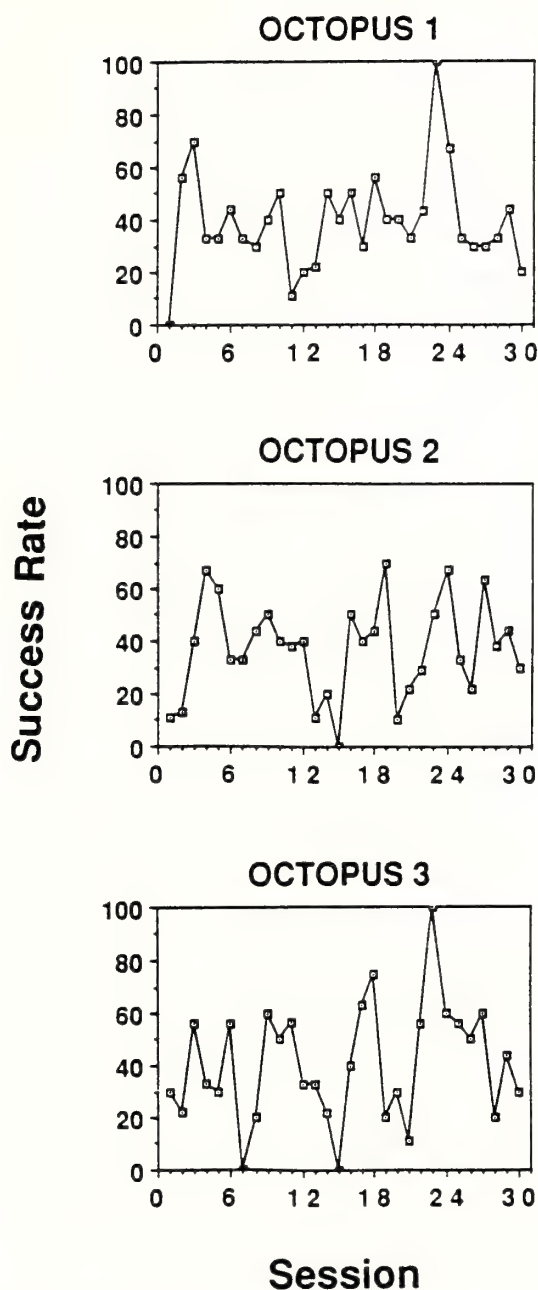


Fig. 7. Mean success rates across sessions for each subject in Experiment II when every combination presented was novel.

by octopuses (Mackintosh, 1965). Now that learning set formation has been indicated in octopuses, experiments with a longer learning set (many more combinations) could make comparisons with vertebrate species possible.

The results from the Experiment II showed no evidence of octopuses learning the concept of oddity. The results were surprising, after the octopuses' promising first day performances towards the end of the learning set. It is possible that more time was needed; the first experiment took place

across three months as opposed to only five weeks for the second experiment. Or, it could be that repeated learning of exemplars, as in the first experiment, could succeed where the repeated novel presentations did not. It also could be that the curtain used during the second experiment prevented cuing that biased the first experiment.

The apparent periodicity in the learning curve for this experiment has no clear explanation. The learning curves for the first experiment also showed temporal variability in performance, especially prior to mastery of the task, but no apparent periodicities. Sanders (1977) has documented octopus learning curves to be multiphasic, corresponding to possible transitions between short- and long-term memory processes. However, his experiments were examining retention of a learned task across hours as opposed to performances of new tasks across days. I expect that the pattern observed in this experiment was simply an artifact of the small sample size and short duration of the experiment.

There were a number of problems in Experiment I. First, response rates were low, perhaps because, with so many trials each day, the smallest practical food rewards still added up to more than their normal daily intake levels. Another related explanation is that older, laboratory-habituated animals seem to have smaller appetites and to be less responsive in general. Second, choices during the first trial of each training session were significantly less accurate, as compared to subsequent trials ($\chi^2 = 22.884$, d.f. = 7, $P < 0.01$). Results from this experiment were therefore conservative. Third, animals were significantly more likely to grab stimuli in the front half of the tank. The experimental design was balanced, however, for front and back placement of the odd object. These three problems appeared to be addressed successfully in Experiment II by switching to a linear presentation, providing simple reinforcement trials at the beginning of each session, limiting trials to ten per day, and using younger, freshly caught animals.

Clearly, octopuses are capable of some forms of complex learning. While it remains to be seen if these octopuses can master a relative class concept such as oddity, these exploratory experiments suggest that the abilities underlying the formation of a learning set have evolved in an invertebrate, as well as in vertebrates (Pearce, 1987). They provide further evidence for convergences in function despite divergences in physiology between invertebrates and vertebrates (Packard, 1972; Corning *et al.*, 1976).

ACKNOWLEDGMENTS

I would like to thank W. M. Kier for sharing his expertise and for use of his laboratory, R. H. Wiley for his encouragement and thoughtful critiques, and A. Smith for his camaraderie in times of aquatic crisis. I would also like to thank my anonymous reviewers for their helpful comments. This research was supported by the University of North Carolina Curriculum in Ecology and Sigma Xi Grants-in-Aid of Research.

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Date of manuscript acceptance: 5 November 1990

APPENDIX 1

Combinations in Experiment I

- Combination 1: *Noetia ponderosa* (Say) (+), *Anomia simplex* Orbigny (-).
Combination 2: *Anomia simplex* Orbigny (+), *Chione cancellata* (Linne') (-).
Combination 3: *Aequipecten gibbus* (Linne') (+), *Mercenaria mercenaria* (Linne') (-).
Combination 4: White square plastic grid (3 x 3 x 1 cm) (+), white square

flat plastic chip (3 x 4 x .4 cm) (-).

- Combination 5: white PVC adaptor fitting, threaded by slip, six-sided middle section (2.9 x 4 cm) (+), white PVC pipe section (4.1 cm diam. x 1.6 cm width) (-).
Combination 6: *Crassostrea virginica* (Gmelin) (+), *Busyon contrarium* (Conrad) (-).

APPENDIX 2

Combinations in Experiment II

Trials in Experiment II consisted of presenting combinations of three shells, two of one species and one of another. Species were chosen randomly with the constraint that the two types of shells of a combination must differ on two of three features (texture: 1=smooth, 2=ridged, 3=pointed; type: 1=gastropod, 2=bivalve or slipper; color: 1=light, 2=dark, 3=patterned).

Species included were: *Aequipecten gibbus* (Linne') (2,2,3); *Amphidromus entobaptus* (Dohrn) (1,1,1); *Anadara brasiliensis* (Lamarck) (2,2,1); *A. ovalis* (Bruguiere) (2,2,1); *A. ovalis* (Bruguiere) (2,2,1); *Anomia simplex* Orbigny (1,2,1); *A. simplex* (1,2,1); *A. simplex* (1,2,2); *Argopecten* sp. (2,2,1); *A. sp.* (2,2,2); *A. sp.* (3,2,3); *A. sp.* (2,2,3); *Arca zebra* Swainson (2,2,3); *Architectonia nobilis* Roding (2,1,3); *Babylonia areolata* (Link) (1,1,3); *Bursa* sp. (3,1,3); *Chicoreus cichoreum* (Gmelin) (3,1,3); *Chione cancellata* (Linne') (2,2,3); *C. cancellata* (2,2,1); *C. cancellata* (2,2,1); *C. cancellata* (2,2,1); *C. paphia* Linne' (2,2,3); *Conus pulcher* Lightfoot (1,1,1); *Crassostrea*

virginica (Gmelin) (1,2,2); *C. virginica* (2,2,1); *C. virginica* (1,2,2); *C. virginica* (2,2,1); *Crepidula fornicata* (Linne') (1,2,1); *C. fornicata* (1,2,1); *C. fornicata* (1,2,2); *C. fornicata* (1,2,1); *Dodinia discus* (Reeve) (1,2,1); *Ficus subintermedia* (Orbigny) (1,1,1); *Geukensia demissa* (Dillwyn) (2,2,2); *Helicostyla* sp. (1,1,3); *Haliotis asinia* Linne' (1,2,2); *Liguus virineus* (Linne') (2,1,3); *Lucina pectinata* (Gmelin) (2,2,1); *Marisa crounarietis* (Linne') (1,1,3); *Melongena corona* Gmelin (3,1,3); *Mercenaria mercenaria* (Linne') (1,2,1); *Murex fulvescens* Sowerby (3,1,1); *Natica* sp. (1,1,1); *N. stellata* Chenu (1,1,1); *Noetia ponderosa* (Say) (2,2,1); *N. ponderosa* (2,2,1); *N. ponderosa* (2,2,2); *Oliva sayana* Ravenel (1,1,1); *O. servicea* Roding (1,1,3); *Phalium granatum* (Born) (1,1,3); *Pitar morrhuana* (Linsley) (1,2,2); *Pleuroplaca* sp. cf. *glabra* (Dunker) (2,1,1); *Sinum perspectivum* (Say) (1,2,1); *Sisula solidissima* (Dillwyn) (1,2,1); *Tagelus plebius* (Lightfoot) (1,2,1); *Tectarius* cf. *Coronatus valenciennes* Gmelin (3,1,3); *Telescopium telescopium* (Linne') (1,1,2); *Tellina alternata* Say (2,2,1); *Trachycardium egmontium* (Shuttleworth) (2,2,1); *T. egmontium* (2,2,2); *Turbo* sp. (1,1,1); *T. sp.* cf. *patholatus* Linne' (1,1,2); *Turritella* sp. (2,1,3); *Vexillum rugosum* (Gmelin) (2,1,3).

Mating behavior of the freshwater pulmonate snail, *Physa gyrina*

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Abstract. The typical mating sequence of freshwater pulmonate snails includes mounting, positioning, preputium eversion, intromission and dismounting. Previous studies report that sperm recipient behavior is passive or absent, and fecundity increases with size. I examined the mating sequence, mate rejection and fecundity of *Physa gyrina* (Say) removed from the Fenway in Boston, Massachusetts. Mate rejection occurred in any of four stereotypical ways: 1) shell swinging; 2) shell jerking; 3) increasing distance to the gonopore; 4) making head/preputium contact. The jerking behavior corresponds to what others described as sham copulation. Shell swinging was similar to parasite escape behavior. Fecundity increased with the size of sperm recipients but decreased with the size of sperm donors. Differences in methodology could explain discrepancies in the interpretation of mating behavior among studies.

Many studies have been conducted on the reproductive biology of freshwater pulmonate snails (Basommatophora; Geraerts and Joosse, 1984). Several behavioral studies, however, have reported conflicting observations within and among closely related taxa. This variation could be due to genetic variation and phenotypic plasticity, as well as differences in research methodology. The most complete information on basommatophoran reproductive behavior is for the sister families Lymnaeidae and Physidae. Physid snails have been studied recently in terms of predator induced life history shifts (Crowl, 1990; Crowl and Covich, 1990), macrophyte herbivory (Sheldon, 1987), sperm storage (Wethington and Dillon, 1991), parasite defense strategies (Townsend and McCarthy, 1980) and mate choice patterns (T. J. DeWitt, unpub. data).

Physa (= *Physella*) *gyrina* (Say) is widely distributed (Clarke, 1981) in lentic environments, especially where pollution or high temperatures exclude other snails (Clampitt, 1970; Harman, 1974). Physid snails, like lymnaeids, are hermaphrodites that usually cross fertilize but are capable of self fertilization (Colton, 1918; R. M. DeWitt and Sloan, 1959). Although the male genital tract can mature briefly before that of the female, it is unclear as to whether physids are ever functionally protandric (Duncan, 1959). Furthermore, snails can receive sperm regardless of the sexual maturity of female tracts, but can not oviposit (Duncan, 1959).

Peak breeding in the field for *Physa gyrina* is reported to occur from April through June in Iowa (Clampitt, 1970) and Michigan (R. M. DeWitt, 1955), although mature snails collected any time of the year will breed in the laboratory as long as water temperature exceeds 10°C (R. M. DeWitt, 1955). This corresponds to the April to May peak reported for *P. fontinalis* Linné by Duncan (1959) at a similar latitude.

In this paper, I report observances taken on the

reproductive behavior of *Physa gyrina* removed from the field. I examined the typical mating sequence, mate rejection, and fecundity. Conflicting reports on mating behavior within and between pulmonate snail species could be due to research methodology.

METHODS

On six independent occasions between February and May 1990, 300-500 snails were collected from the same location in the Fenway in Boston, Massachusetts (NW of Beth Israel Hospital). Snails were collected between 1200 and 1600 hours. Dip nets were used to collect litter from the bottom of the Fenway (1-1.5 m depth). Snails were removed from the litter, by hand, to a bucket containing Fenway water. On all occasions, *Physa gyrina* made up over 98% of snail fauna [other species, in order of abundance, included *Planorbella campanulata* (= *Helisoma campanulatum*) (Say) and *Stagnicola elodes* (= *Lymnaea palustris*) (Say)]. Each collection, including all specimens, was brought back to the laboratory and placed in an aerated, filtered 38 l aquarium with approximately 20 l of water (half from the collection site, the other half was conditioned tap water). Population densities were greater than, but similar to those observed in the field during collection. Snails were kept at 20°C near a natural light source. They were fed canned or boiled vegetables (carrot, pea, chick pea). This diet was supplemented by unidentified algae in the aquaria.

Preliminary observations of snails were made to define criteria for successful versus failed copulation. Criteria I used to define successful mating were that 1) the sperm donor was in place at the shell margin of the recipient; 2) the preputium was placed in the immediate area of the gonopore; 3) the seminal vesicle within the preputium contained a milky

substance; 4) no agonistic behavior occurred within the first 30 seconds of mating.

TREATMENT 1

After approximately 18 hours from the time of collection, interacting *Physa gyrina* pairs were observed. As soon as the nature of each interaction was determined, pairs were removed and measured from the shell apex to the most distal portion of the shell margin. Sperm recipients from successful matings were removed to mason jars for a week and the number of eggs they laid was recorded. This continued until 12 successful matings were observed on each of the six occasions (72 observations total).

TREATMENT 2

An additional 90 *Physa gyrina* pairs were observed as in treatment one. In treatment two, however, pairs were not interrupted, but allowed to finish their interactions.

RESULTS

The sizes of sperm donors and recipients in treatment one were 7.32 ± 0.96 and 7.90 ± 1.12 (mean $\pm$ sd), respectively. The generalized mating sequence for *Physa gyrina* was similar to that described for *Lymnaea stagnalis* (Linne') (van Duivenboden and Maat, 1988), a closely related taxon, except that it occurred more rapidly. The sequence of successful mating included mounting (mate selection?), positioning, eversion of the preputium, intromission and dismounting. This process was sometimes interrupted by mate rejection but lasted approximately 10-20 minutes when successful (Fig. 1).

Rejection of mates was observed to occur in four

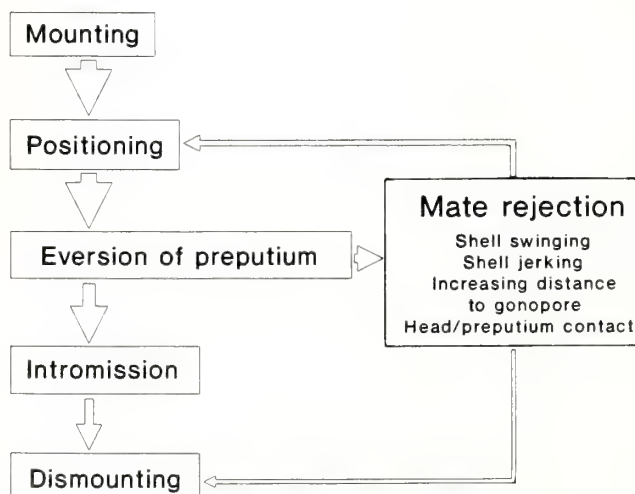


Fig. 1. Mating sequence of *Physa gyrina*. Thickness of arrows approximates the frequency that path is followed.

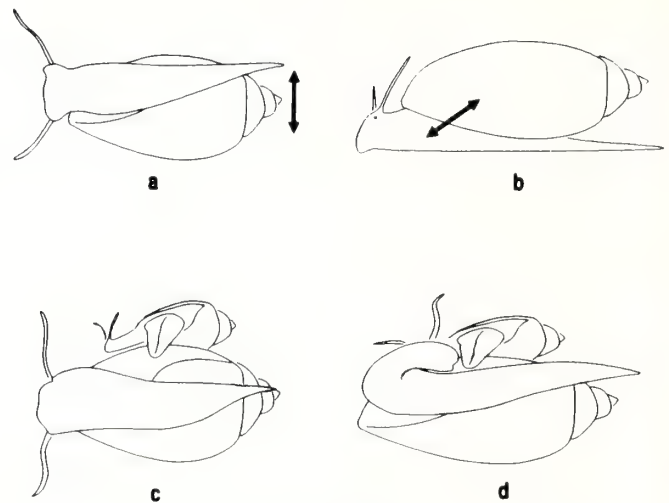


Fig. 2. Stereotypical mate rejection behavior by sperm recipients. a) shell swinging, b) shell jerking, c) increasing distance to gonopore, d) head/preputium contact.

stereotypical ways (Fig. 2). In order of decreasing frequency, intended sperm recipients performed 1) shell swinging, 2) shell jerking, 3) shell positioning such that sperm donors could not reach the gonopore, 4) head to preputium contact ("biting").

Shell swinging was characterized by a 180-270° left and right twisting of the shell about the stationary foot. Shell jerking was rapid contraction of the shell toward the foot, with gradual release. This was generally repeated several times until the preputium was withdrawn. Shell positioning by intended sperm recipients occurred such that the distance from their shell margin to their gonopore was increased, effectively placing the gonopore out of reach of the sperm donor's preputium. I could not observe whether head to preputium contact included radular action but think it likely given that physid snails rarely cease radular movement and that the reaction from sperm donors was always immediate, violent withdrawal of the preputium, usually followed by immediate dismount. The first three forms of agonistic sperm recipient behavior generally resulted in two or three re-attempts at mating by sperm donors before they dismounted.

I commonly observed chain copulations of up to five snails, acting alternately as sperm donors and recipients. Furthermore, triads were observed on three occasions while collecting in the field. Polyandrous copulation, which has only been clearly described (among basommatophorans) for *Physa fontinalis* (Duncan, 1959), was not observed.

The range of snails observed to oviposit in this study was 6.3-10.9 mm. Larger sperm recipients proved significantly more fecund (R^2 for log transformed data = 0.58, $p_{\alpha/2} = 0.0001$). However, the size of sperm donors was negatively correlated with sperm recipient (log transformed) fecundity (pearson correlated coefficient = -0.44, $p_{\alpha/2} < 0.01$), even

when the size of sperm recipients was controlled for (partial correlation coefficient = -0.34, $p_{\alpha/2} = 0.025$).

DISCUSSION

Describing the mating behavior of freshwater gastropods can be accomplished by field or laboratory studies. The former may be made difficult by turbid water, detritus, invasiveness of techniques used to locate snails and low probability of finding mating pairs. For these reasons, laboratory investigation is usually employed. This is acceptable if it is reasonable to believe organisms are behaving as they would in the field. Observations from field studies generally concur with laboratory findings (e.g. R. M. DeWitt, 1954b). However, it is likely that the laboratory techniques employed affect the behavior of animals being studied, and account for some of the variation in reported behavior.

R. M. DeWitt (1954a) reported 7 mm as the minimum size at which *Physa gyrina* would oviposit. However, this research of *P. gyrina*, and studies on other physids, suggest that the size at first oviposition varies among populations and species (Duncan, 1959; Clappitt, 1970; McMahon, 1975; Crowl, 1990). This variation could be due to physical variables such as temperature (McMahon, 1975) and plastic life history strategies. *P. virgata* (Gould) (Crowl, 1989; Crowl and Covich, 1989) is induced, by the presence of crayfish (predators), to delay reproduction until larger size is attained. I have observed similarly varied life history patterns in *P. heterostrophus* Say in streams with and without goldfish in New York (unpub. data).

R. M. DeWitt (1954a) reported a lack of chain copulations in *Physa gyrina*. However, I observed chain copulation in the laboratory and field. Chain copulations have been reported for laboratory populations of several other freshwater pulmonates (e.g. Barraud, 1957; Duncan, 1959).

My observation of increasing fecundity as a function of snail size concurs with work on several freshwater pulmonates (e.g. R. M. DeWitt, 1954b; de Wit, 1955; Hunter, 1975; McMahon, 1975). However, this is the first report that the size of sperm donors is negatively correlated with the fecundity of sperm recipients. It will be interesting to see if studies employing different methods obtain similar results. The data from the present study do not allow me to address why larger sperm donors could have suppressed sperm recipient fecundity.

Many studies report that females are passive during mating (R. M. DeWitt, 1954a; Duncan, 1959). Van Duivenboden and ter Maat (1988) state "characteristic female mating behavior is absent". I observed several stereotypical female behavior patterns which I interpret to be mate rejection (Fig. 2). Two of these correspond to behavior reported by other investigators. Shell swinging is similar to weak parasite avoidance reported for *Physa fontinalis* in response to

chemicals from predatory leeches (Townsend and McCarthy, 1980). Shell jerking is similar to the description of "false coupling" given by Barraud (1957) and "sham copulation" given by van Duivenboden and ter Maat (1988) for *Lymnaea stagnalis*. Barraud (1957) describes false coupling as when the female gonopore remains unoccupied while the preputium is bent around her shell margin, despite contorted retractions of the female. Van Duivenboden and ter Maat describe sham copulation as "characterized by strong withdrawal of the forepart of the female, after which she relaxes again...while the preputium remains in place (under the female shell margin — not in the gonopore)." Barraud (1957) report that males may move away without copulation after this event and that copulation was seldom successful. Van Duivenboden and ter Maat (1988) report that nearly all copulations were eventually successful but "sham copulations occur frequently ($\geq 50\%$ of the pairs)".

I believe the phrase "sham copulation" could be a misnomer because the interpretation as mate rejection is strongly supported in this study and that of Barraud (1957). Natural selection should provide strong selection pressure for successful copulatory mechanisms between conspecifics (Ridley, 1983) unless there is a benefit to unsuccessful mating behavior. Let us examine potential costs and benefits of sham (or unsuccessful) copulation. The only benefit I could associate with sham copulation would be the ethological argument that the practice is valuable for future "true" copulation. Noland and Carriker (1946) show that prior experience increases copulatory success in *Lymnaea stagnalis*. However, later studies by Barraud (1957) and van Duivenboden and ter Maat (1988) refute this. Costs of "sham copulation" could go beyond time and energy expenditure. Predation risk may be greater for animals in a pair versus solitary animals [e.g. *Gammarus pulex* (Linneé); Ward, 1986]. Thus, the value of sham copulation is dubious. I suspect that the long period of isolation used in many studies made sperm donors less willing to give up copulation attempts, thereby reducing the occurrence of successful mate rejection and delaying or preventing straightforward copulation.

Researchers often use the isolate-unite technique (e.g. Noland and Carriker, 1946; Barraud, 1957; Duncan, 1959; van Duivenboden and ter Maat, 1988) to study the reproductive behavior of gastropods. This protocol is effective for stimulating copulation, but could lead to overlooking phenomena of more realistic situations for field populations (e.g. mate choice). The protocol could introduce artifact problems as well [e.g. sperm deprivation during isolation eventually leads to initiation of self fertilization (van Duivenboden, 1983), thereby reducing receptivity to mating].

It is probable that a more generalized view of mating behavior in gastropods can be obtained by supplementing the isolate-unite technique with studies using multiple wild-caught snails in as close conditions to their natural environment as

possible (i.e. population density, water, temperature, light, etc.). The use of focal pair sampling (perhaps videotaping) would also be more likely to yield valuable details on mating over multiple pair observation (e.g. van Duivenboden and ter Maat, 1988). The suggested techniques allow elucidation of structured mating, mate rejection and other phenomena occurring in wild populations.

ACKNOWLEDGMENTS

This research was made possible by academic support from Dr. Fred A. Wasserman, to whom I am greatly obliged.

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Date of manuscript acceptance: 22 January 1991

Reproductive patterns and seasonal occurrence of the Sea Hare *Aplysia brasiliana* Rang (Gastropoda, Opisthobranchia) at South Padre Island, Texas

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Abstract. Monthly collections of *Aplysia brasiliana* Rang were made at South Padre Island, Texas over an 18 month period. Seasonal changes in distribution of weight classes support the existence of a maximum life cycle of approximately 1 year. Large numbers of juveniles in spring collections are produced by the reproductive activities of overwintering adults or possibly recruitment of larvae from other areas. The spring recruitment is followed by the disappearance from the population by large overwintering specimens. Spring juveniles increase in size and weight by late summer. Early fall collections yielded no specimens. A minor period of secondary recruitment can occur during late fall or early winter. Winter collections, when successful, yielded reduced numbers of large adults.

A number of investigations conducted on naturally occurring aplysiid populations have centered upon duration of life cycles, seasonal changes in size or weight, reproductive activities, and recruitment into the population by recently metamorphosed juveniles (Miller, 1960; Carefoot, 1967; Usuki, 1970; Audesirk, 1979; Sarver, 1979; Gev *et al.*, 1984). Following a review of these studies, Carefoot (1987) concluded that, while considerable variation does exist in some species, most sea hares do, in fact, exhibit an annual life cycle.

This conclusion is consistent with current information for the sea hare *Aplysia brasiliana*, which is common to the Gulf of Mexico (Strenth and Blankenship, 1978a). For Florida populations of *A. brasiliana*, both Krakauer (1969) and Hamilton *et al.* (1982) concluded that this species exhibits a life cycle of approximately one year. Krakauer's (1969) conclusions relative to *A. brasiliana* (as *A. willcoxi* Heilprin) were, however, based upon only a one year series of collections of relatively small sample sizes. The study by Hamilton *et al.* (1982) was based upon large sample sizes but unfortunately was limited to the months of March through June. Krakauer (1969) also characterized the life cycle of *A. brasiliana* in Florida as having "two waves of settling" with a major "spawning period" in late March and early April. Following several years of preliminary field work, this study was undertaken in an effort to clarify the life cycle of *A. brasiliana*.

METHODS

Collections were made on a continuous monthly basis

in the south Laguna Madre at South Padre Island, Texas, at depths of < 1 m, from July, 1977 through December, 1978. All known habitats of *Aplysia brasiliana* were established during preliminary field work in 1975 and 1976. While each of these habitats was surveyed during each trip, collection techniques and success varied with the season. Winter and spring collections were most productive during early morning low tides. These low tides trapped nocturnally active specimens in shallow grass flats which are common along the western coastal margin of the island. Recently metamorphosed juveniles were collected in the spring from artificial habitats of broken concrete, brick and stone, which had been deposited near the east abutment of the old causeway. During summer months, swimming specimens were collected with dip nets at night near the lights of fishing piers located along the western side of South Padre Island. Specimens were also found on rocks of the boat slip at the Coast Guard Station and the channel side of the north jetty. Following collection, specimens were weighed to the nearest gram.

RESULTS AND DISCUSSION

DURATION OF LIFE CYCLE

The results (Fig. 1) of this study support the existence of a maximum life cycle of approximately 1 year for *Aplysia brasiliana* at South Padre Island, Texas. Summer collections, such as those of 1977, were generally characterized by the presence of specimens in the 50 to 300 gm weight range. These animals increased in size and weight during the late fall and winter. By March of the following spring (Fig. 1),

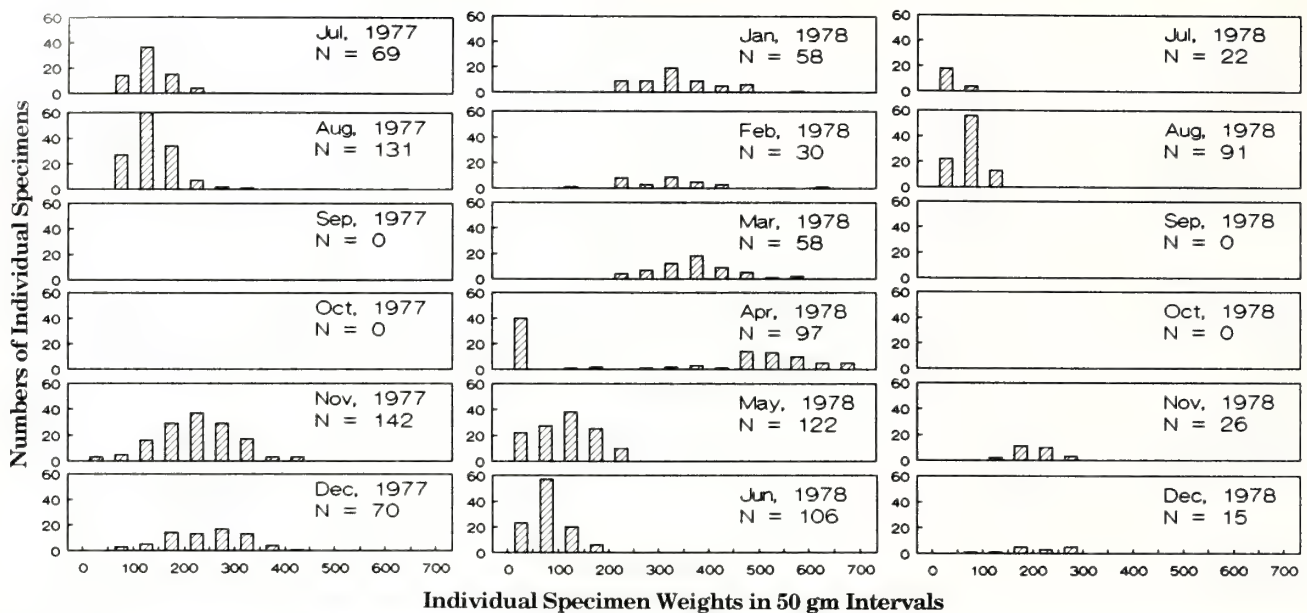


Fig. 1. Numbers of specimens of *Aplysia brasiliana* in each 50 gm weight class of monthly collections at South Padre Island, Texas from July, 1977 through December, 1978.

the population was characterized by specimens in the 250 - 600 gm range. The marked appearance of large numbers of recently recruited juveniles in the April, 1978 collection, as well as moderate numbers of large specimens in the 450 - 700 gm size, clearly revealed the overlap of generations. These very large specimens were absent from the population by the following month. Similar life cycle lengths have been reported by Carefoot (1967) for *A. punctata* Cuvier, by Audesirk (1979) for *A. californica* Cooper, and by Usuki (1970) for *A. kurodai* (Baba) and *A. juliana* Quoy and Gaimard.

RECRUITMENT

The results of this study (Fig. 1) confirm the presence of a major period of recruitment of recently metamorphosed juveniles during the spring of the year. The April, 1978 collection clearly revealed the marked appearance of large numbers of specimens in the 1 - 50 gm weight class. The presence of specimens weighing less than 50 gm in the collections of June, July and August of 1978 initially suggested a continuous 5 month period of recruitment. This does not, however, appear to be the case. Based upon field observations made during 1975, 1976 and 1977, the spring recruitment of 1978 appeared unusually large. Hundreds of swimming specimens were observed in May. Small to medium sized specimens were very abundant on the rocks of the jetty and Coast Guard Station.

A marked decline in numbers of specimens was evident by early summer and, despite an extensive collecting effort, the July collection resulted in only 22 specimens. It appears that the unusually large spring recruitment of 1978

was followed by a mid-summer crash in the population. While the June, July and August collections were characterized by specimens under 50 gm, there were no specimens in the 1 - 10 gm range which had characterized the April collection. The small specimens present in the June, July and August collections of 1978 were therefore considered to be spring recruits which were unable to increase in size due to competition, rather than recent recruits to the population. Collections made during the summer of 1977 (Fig. 1) as well as those of 1976, appear to support this conclusion. These collections were characterized by specimens in the 50 - 300 gm range, as well as the absence of smaller specimens in the 1 - 50 gm range. The summer collections of 1977 are therefore considered typical for this species.

The presence of one 12 gm specimen as well as several others weighing less than 50 gm in the November, 1977 collection (Fig. 1) supports the existence of a minor period of secondary recruitment during the late fall or early winter. The life cycle of *Aplysia brasiliana* at South Padre Island appears to be characterized by the presence of a major period of spring recruitment followed by a very minor period of secondary recruitment during late fall or early winter. Usuki (1970) reported similar findings for *A. kurodai* in the Sea of Japan.

Aplysia brasiliana appears to exhibit a relatively high reproductive potential during much of its life cycle. Upon capture, specimens were observed to engage readily in copulatory and egg laying activities throughout most of the year. Despite this fact, recruitment is clearly not a continual process. Recent life history studies (e.g. Sarver, 1979; Gev *et al.*, 1984) have established the relationship of the seasonal abundance

of select algal species with the timing of metamorphosis and subsequent recruitment of juveniles into naturally occurring populations of various aplysiid species (see Carefoot, 1987, for review).

Mature veliger larvae of *Aplysia brasiliana* readily metamorphose in the presence of the red alga *Callithamnion* and, to a lesser degree, *Polysiphonia* (Strength and Blankenship, 1978b). While *Callithamnion* can be found throughout most of the year (Sorensen, 1979), it reaches its maximum abundance at South Padre Island during March (Penn, 1974). This seasonal abundance of *Callithamnion* just precedes the major period of recruitment of juvenile *A. brasiliana* into the population in April. While many additional environmental factors should be considered, it appears possible that this seasonal peak of *Callithamnion* could be a major contributing factor in the timing of the spring recruitment of juvenile *A. brasiliana* at South Padre Island, Texas.

SEASONAL OCCURRENCE

The disappearance of specimens from otherwise normally occupied habitats during the months of September and October (Fig. 1) appears to be a normal aspect of the life cycle of *Aplysia brasiliana* at South Padre Island. Krakauer (1969) also failed to collect specimens of this species in Florida during this same time period. Studies on other aplysiids have reported similar decreases in numbers of specimens during the fall of the year. Audesirk (1979:413) reported a "nearly total disappearance of animals" for *A. californica* during October, November and December. Gev *et al.* (1984:69) reported that "The *Aplysia* season ends in September" for *A. depilans* Gmelin and *A. fasciata* Poiret along the Mediterranean coast of Israel. Both Audesirk (1979) and Gev *et al.* (1984) related this noticeable drop in numbers of specimens during the fall of the year to the decline of one year class, which in turn is replaced by the succeeding generation.

Results obtained during the course of this study do not support the above premise as it relates to *Aplysia brasiliana* at South Padre Island. While some minor recruitment could occur during November or December following the absence of collectable fall specimens, there is no major shift in modal weight class during the fall such as that observed for the month of April, 1978 (Fig. 1). In addition, average weights of specimens increased from August to November during both years. The average weight during 1977 increased from 139 gm (N = 131) in August to 220 gm (N = 142) in November. During 1978, the average weight increased from 67 gm (N = 91) in August to 206 gm (N = 26) in November. The demonstrated presence of continual weight increase in specimens from August to November, as well as the absence of large numbers of recently recruited juveniles in the early winter collections, serve to support the existence of a

standing population of *A. brasiliana* which is characterized by slight to moderate increases in size of individual specimens rather than a population undergoing decline and replacement.

A suitable explanation to account for the location of specimens during September and October does not appear forthcoming from observations made during the course of this study. While discounting reproductive migration in general, Carefoot (1987:204) states that the theory of feeding migrations is often "attractive in that it accounts for seasonal gaps in abundance." Neither the theory of reproductive migration nor that of feeding migration appear to provide a feasible explanation (see below) to account for the disappearance of *Aplysia brasiliana* at South Padre Island during the early fall of the year.

Aplysia brasiliana is a known burrower (Aspey and Blankenship, 1976). Individual specimens could be undergoing continual or prolonged intermittent periods of burrowing in the soft substratum of the south Laguna Madre during September and October. It should be emphasized that this hypothesis is conjectural and not supported by field work. It should be noted, however, that decreased foraging activities by burrowed *A. brasiliana* during September and October could possibly facilitate a rebound in the standing crop of *Callithamnion*, which in turn could account for the secondary period of recruitment during late fall or early winter.

MIGRATION

In his review of migration theory as it relates to life cycles of aplysiids, Carefoot (1987:203) states that "This theory of migration has fallen into disfavor from lack of supporting evidence". While individual specimens were observed to exhibit localized movements in association with foraging behavior, the current study provides no support for the migration theory as it relates to *Aplysia brasiliana* at South Padre Island, Texas. This could be due in part to the somewhat confined nature of the south Laguna Madre as well as the presence in the lagoon of both adult food (*Laurencia* and *Gracilaria*) as well as metamorphosing substrata (*Callithamnion* and *Polysiphonia*) for developing larvae. Juveniles and adults were collected from the exact same habitats during different times of the year.

While specimens of *Aplysia brasiliana* are found occasionally beached on the Gulf of Mexico side of the island, the nature of the offshore substrate does not appear to favor the attachment of suitable benthic marine algae. Consequently, the immediate offshore environment of South Padre Island appears to offer little if any favorable habitat for completion of all or part of the life cycle of *A. brasiliana*. While not surveyed in this study, deeper off-shore reefs are known habitats (Tunnell and Chaney, 1970) for *A. brasiliana* and could provide for variations in life cycles not observed during the course of this study.

CONCLUSIONS

The results of this study are consistent with and support the conclusions of both Krakauer (1969) and Hamilton *et al.* (1982) that the maximum length of the life cycle of *Aplysia brasiliiana* is approximately one year. This species exhibits a major period of recruitment during the spring followed by a marked loss of large overwintering specimens from the population. A minor period of secondary recruitment can occur during late fall or early winter. The reason for the absence of collectable specimens during the early fall currently remains obscure.

ACKNOWLEDGMENTS

The presentation of the results of this study during the American Malacological Union meeting at Woods Hole was made possible by a Faculty Development Grant to one of us (N.E.S.) from Angelo State University. Field studies were supported by a National Science Foundation grant (BBS 8711368) to one of us (J.E.B.). Appreciation is extended to the faculty and staff of the Pan American University Marine Laboratory at South Padre Island, Texas. This study would not have been possible without their support. A very special thanks goes to Barbara Strenth for preparation of the figure.

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Date of manuscript acceptance: 21 December 1990

Variation in sense organ design and associated sensory capabilities among closely related molluscs

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Abstract. Knowledge of ontogenetic and interspecific variation in structural and functional properties of an organ is prerequisite to establishing valid generalizations about the organ's role within a group. However, for many molluscan sense organs, generalizations have become established with minimal knowledge of such variation. Review of data on the gross structure, optical properties and visual responses of 44 gastropods provides a clear example of this problem. Lens structures range from crystalline to gelatinous, with concomitant differences in refractive index, and degree of structural and optical homogeneity. Refractive index measurements from differential interference microscopy indicate that gastropod lenses could be partly or completely corrected for spherical aberration. Photoreceptor separation distances vary from about 3 to 25 μm , and photoreceptor abundances from about five to 100,000 per eye. Estimates of anatomical resolution vary from 0.25 to 14 degrees, and overlap considerably with values for arthropods and vertebrates. Visual responses range from simple taxis to the ability to detect an object's orientation. Reviews of data on the structure and function of opisthobranch rhinophores, and on scallop eye structure, also reveal greater variation than is typically appreciated. More attention must be given to variation in molluscan sense organ structure and function in order for this field to develop more fully.

The field of molluscan biology has been expanding its horizons over the past quarter century from its earlier emphasis on studies of taxonomy, shell morphology and anatomy (Solem, 1974), to greater exploration of life histories, ecological relationships, and behavior. Although few scientists with formal training in animal behavior specialized in studying molluscs 20 years ago, this is no longer true. Most animal behaviorists are still attracted to the more rapidly moving arthropods and vertebrates, but cephalopods have always presented a unique challenge, and the value of certain gastropods as neuroethological models has attracted numerous neurobiologists and some behaviorists, especially those interested in a reductionist approach. A computer search of literature citations dealing with the behavior of molluscs clearly demonstrates the rapid expansion of this subdiscipline (Fig. 1). More than 7,600 citations in Biological Abstracts have included information about molluscan behavior or sensory biology over the past 20 years, and the current rate of increase is about 500 citations per year.

The usual pattern of development in many biological subdisciplines is for initial studies to be conducted on one or two easily obtained species thought to be typical of a group, and for initial generalizations to be developed for the group based on the findings obtained for these 'type' species. Later, as is required for any field to mature, significant efforts must be directed toward consideration of a broad range of species. Information from comparative studies provides opportunities

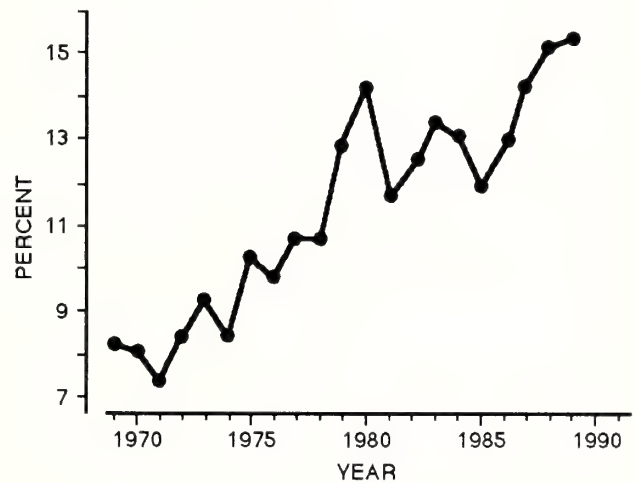


Fig. 1. Number of publications dealing with the behavior or sensory biology of molluscs, as a percentage of all publications dealing with molluscs, over the past two decades. These data are based on a computer search of Biological Abstracts, and should be considered only an approximation of the general trend.

to determine the phylogenetic limits of plasticity in the group, and often leads to revision of generalizations for the group and reconsideration of whether the initially studied species are indeed typical of the group.

Despite the substantial body of information already acquired on the behavior and sensory biology of molluscs, this

knowledge base is limited in several critical ways. Much of the information contained in the 7,600 citations noted above concerns just a handful of species, and the levels of intrageneric and intrafamilial variation have been hardly examined. In other cases intragroup variation has been examined and found to be considerable, but this does not seem widely appreciated. And finally, some of the 'type' species recognized by early investigators of molluscan behavior and sensory biology do not seem particularly representative in the context of the broader base of knowledge available today. Collectively, these factors would limit the validity of generalizations made about the behavior and sensory biology of any animal group. However, this is especially true for molluscs, as they have undergone an extensive adaptive radiation into a wide range of habitats, and they exhibit substantial plasticity in sense organ structure and behavior (Seed, 1983; Audesirk and Audesirk, 1985).

It seems, therefore, that substantially more attention needs to be given to the variation that exists within molluscan groups in the areas of behavior and sensory biology in order to resolve these problems and allow this field to achieve a more mature level of development. The goal here is to convince the reader of that viewpoint by examining three selected examples involving a range of taxonomic levels. First, eye structure and the behavioral function of vision in the entire Class Gastropoda will be reviewed in some detail. Then, briefer comparisons will be made of the structure and apparent behavioral function of a pair of head tentacles termed 'rhinophores' in the gastropod Subclass Opisthobranchia, and of the structure of the eyes in the bivalve Family Pectinidae (scallops).

GASTROPOD EYES AND VISUALLY MEDIATED BEHAVIOR

The primary example involves vision in gastropod molluscs. It has been traditional for zoologists (e.g. Messenger, 1981) to recognize three structural grades of eyes among the Gastropoda:

- a) the open cup or pit eye, in which the intraocular space is unfilled and not isolated from the surrounding medium by a cornea (e.g. *Patella*);
- b) the filled cup eye, in which a gelatinous (and hence low refractive index) material fills the intraocular space, but in which a cornea is lacking (e.g. *Haliotis*);
- c) the closed cup eye, in which a soft or hard lens is present in the intraocular space, and in which a cornea is present (e.g. *Nerita*, *Strombus*, *Littorina*).

Recognition of three grades of structure in gastropod eyes can describe, at best, but three points within what is a wide range of ocular designs. Furthermore, because two of the three traditionally recognized grades are exhibited only by quite primitive gastropods (e.g. *Patella*, *Haliotis*), this three

grade scheme cannot reflect adequately the range of variation present in gastropod eyes.

Land (1981, 1984) and Cronin (1988) have reviewed certain aspects of eye structure and optical properties in invertebrates, or particularly in molluscs, and limited efforts have been made to tabulate data on gastropod eyes (Zunke, 1978; Messenger, 1981; Land, 1984). Although many excellent studies have been made of retinal ultrastructure and neurophysiological responses, these studies have tended to provide incomplete or no information about whole eye structure or, importantly, the eye as a complete optical system. Thus, there are fewer published data than some might suspect on which to base any general conclusions about gastropod vision. A more detailed comparative view of eye structure in this group is provided here (Table 1) by pooling most of the published data, which covers about 32 species, with previously unpublished data on an additional 12 species.

Few authors who have studied gastropod eyes have reported retinal surface areas, or photoreceptor separation distances, abundances, and densities. Thus, most of the data in Table 1 are derived from my computations based on illustrations and data included in the references cited. Average intraocular diameter is the mean of the major axis (distance from pupil to retina along optic axis) and the minor axis (distance between opposite retinal surfaces along a line perpendicular to the major axis and mid-way along it). Retinal areas were computed based on an ellipsoidal model, using the major and minor axes described above, with subtraction of that portion of the ellipsoid's total surface area corresponding to the aperture, where no photoreceptors are located. In most cases, photoreceptor separation distances were obtained directly from an author's measurements or illustrations. However, for *Pterocera* (= *Lambis*) *lambis* (Linné) and *Aplysia californica* Cooper, the separation distances used here are 1 μm greater than the indicated photoreceptor diameters; this adjustment corresponds to a minimal thickness of supportive cells separating adjacent receptors. Receptor knowledge base is limited in several critical ways. Much of the information contained in the 7,600 citations noted above concerns just a handful of species, and the levels of intrageneric and intrafamilial variation have hardly been examined. In other cases intragroup variation has been examined sides whose length equals the receptor separation distance). A hexagonal model matches the actual spatial positions of photoreceptors seen in ideal sections taken tangential to the inner retinal surface, for a variety of gastropods (e.g. Hamilton *et al.*, 1983). Receptor densities were computed from retinal area and receptor abundance values. For lens sizes, a single value is given for spherical lenses, and the order of the two values listed for oblong lenses represents the lengths parallel and perpendicular to the optic axis, respectively. Shell length was used as a measure of body length, except in species where the shell is clearly reduced or absent. The length

Table 1. Variation in eye structure among selected gastropod molluscs.

Taxon	Adult Length (mm)	Mean Intraoc. Diam. (μm)	Retinal Area (mm ²)	Receptor Separ. Dist. (μm)	Receptor Abund.	Receptor Density (mm ⁻²)	Lens Diam. (μm)	Reference/Source
PROSOBRANCHIA								
<i>Haliotis discus</i> Reeve	150	840	1.703	10.0	19665	11550	n/a	Tonosaki (1967)
<i>Turbo castanea</i> Gmelin	16	514	0.555	2.7	89840	161980	385+	a
<i>Neritina reclivata</i> (Say)	15	248	0.161	5.7	5735	35540	235+	a
<i>Littoraria irrorata</i> (Say)	19	233	0.109	4.4	6565	60470	170	Hamilton <i>et al.</i> (1983)
<i>Littorina littorea</i> (Linne')	22	143	0.052	12.0	415	8030	110	Newell (1965)
<i>Tectarius muricatus</i> (Linne')	19	178	0.074	4.5	4210	57030	141	a
<i>Strombus luhuanus</i> Linne'	40	1184	3.025	6.0	97020	32080	732	Gillary (1974), Gillary and Gillary (1979)
<i>Pterocera lambis</i> (Linne')	90	1023	2.893	12.0	23200	8020	1023	Prince (1955)
<i>Lioplax pilsbryi</i> Walker	28	220	0.121	5.5	4610	38210	165x150	a
<i>Pomacea paludosa</i> (Say)	50	561	0.775	4.0	55940	72170	440x322	a
<i>Elimia curvicostrata</i> (Reeve)	15	103	0.028	4.5	1600	57030	112x104	a
<i>Marginella</i> sp.	9	120	0.031	6.5	835	27330	102	a
<i>Nassarius vibex</i> (Say)	13	197	0.085	6.9	2050	24250	150x165	a
<i>Melongena corona</i> (Gmelin)	76	273	0.166	7.9	3075	18510	215x175	a
OPISTHOBRANCHIA								
<i>Bulla gouldiana</i> Pilsbry	50	327	0.285	12.5	2110	7390	300x225	Jacklet and Colquhoun (1983)
<i>Aplysia brasiliana</i> Rang	178	507	0.613	16.0	2765	4510	388x297	a
<i>A. californica</i> Cooper	216	471	0.572	16.0	2585	4520	450x400	Jacklet and Geronimo (1971), Herman and Strumwasser (1984)
<i>Hermisenda crassicornis</i> (Eschscholtz)	—	—	—	—	5	—	35x50	Stensaas <i>et al.</i> (1969)
<i>Tritonia diomedea</i> (Bergh)	—	—	—	—	5	—	150x130	Chase (1974)
Mean for 16 nudibranchs	—	—	—	—	8	—	57	Hughes (1970)
PULMONATA								
<i>Melampus bidentatus</i> Say	13	101	0.018	7.4	375	21210	63x100	a
<i>Lymnaea stagnalis</i> (Linne')	28	140	0.050	10.0	585	11610	110x100	Stoll (1973)
<i>Biomphalaria glabrata</i> (Say)	17	212	0.121	14.8	640	5290	163x131	Schall and Baptista (1990)
<i>Strophocheilus</i> sp.	120	350	0.338	23.8	690	2040	291x240	Oswaldo-Cruz and Bernardes (1982)
<i>Euglandina rosea</i> (Férussac)	63	177	0.083	5.0	3825	46210	175x150	a
<i>Helix aspersa</i> Müller	36	231	0.134	6.3	3890	29120	225x200	Eakin and Brandenburger (1975), Brandenburger (1975)
<i>Succinea putris</i> (Linne')	17	97	0.026	14.7	145	5490	60x70	Zunke (1978)
<i>Limax flavus</i> Linne'	88	209	0.132	15.0	680	5160	145x175	Kataoka (1975, 1977)
<i>Agriolimax reticulatus</i> Müller	43	94	0.013	20.0	40	3150	87x67	Newell and Newell (1968)

+ = Additional lens protrusion through aperture. a = Based on author's previously unpublished observations.

values used were those stated in the references indicated in Table 1 or, when authors failed to indicate the sizes of the animals studied, those given as average adult sizes in appropriate basic references.

Starting at the largest scale, one can consider the relative sizes of the eyes in adults of various species. Figure 2 shows the relationship between the mean diameter of the intraocular space and the body length of adults for 26 species distributed among three subclasses. Two points are clear from this analysis. First, the size of the intraocular space varies fairly widely among gastropods, with *Strombus luhuanus* Linne' having an average intraocular diameter more than 12 times greater than *Agriolimax reticulatus* Müller. Second, average intraocular diameter is significantly correlated with

body length when all three subclasses are pooled together ($r=0.43$, $P=0.030$), and for the subclasses Prosobranchia ($n=14$, $r=0.62$, $P=0.019$) and Pulmonata ($n=9$, $r=0.75$, $P=0.019$) when analyzed separately. Opisthobranchs were not analyzed separately because reasonable measures of intraocular diameter are available for only three species. Gastropod eyes can be positioned within a substantial peri-optic sinus, as in littorinids (Newell, 1965; Hamilton *et al.*, 1983), or they can be closely surrounded by connective tissue, as in *Aplysia californica* (Herman and Strumwasser, 1984).

As would be expected, lens size (as measured by area in mid-sagittal section) is highly correlated with average intraocular diameter ($r=0.87$, $P<0.0001$) and the amount of

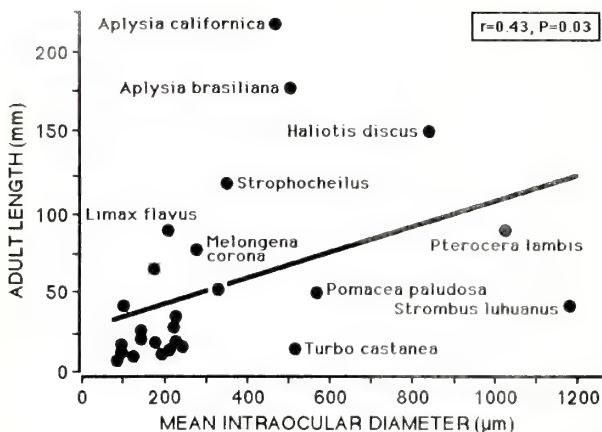


Fig. 2. Relationship between the mean diameter of the intraocular space and the adult body length of 26 gastropods from three subclasses. The species associated with the outlying points are identified. Significant positive correlations also exist for just the prosobranchs ($n=14$) and just the pulmonates ($n=9$).

retinal surface area ($r=0.97$, $P<0.0001$). The abalone *Haliotis discus* Reeve lacks a lens, as reportedly do *Trochus* and *Patella* (Hilger, 1885), but the eyes of all other gastropods surveyed here have a lens or lens-like material located in the intraocular space. Optical properties of lenses are influenced partly by their shape. Lens shapes vary from spherical or nearly so, as in the littorinids, strombids and *Lymnaea*, to ellipsoidal, as in various prosobranchs, the aplysiids and most pulmonates (Table 1). Some amount of vitreous material is present between the lens and retina in most species, although Dorsett (1986) suggests this is not the case. Past confusion concerning the presence or absence of vitreous material in the eyes of various species could have been due to the susceptibility of vitreous material to dissolution during histological processing (Charles, 1966; Hamilton *et al.*, 1983).

The lens of *Aplysia californica* was originally illustrated as being spherical, and filling the entire intraocular space (Jacklet *et al.*, 1972). This report led logically to the conclusion that its eye could not form a sharp image (Messenger, 1981; Dorsett, 1986) because of insufficient distance for light rays to be brought to focus on the retina (Land, 1981). However, the illustration in Jacklet *et al.* (1972) is based apparently on an earlier section of the eye in which no aperture was visible, indicating that the section was oblique rather than longitudinal (Jacklet, 1969). A more recent study by Herman and Strumwasser (1984) has shown clearly that the *A. californica* lens and intraocular space are quite ellipsoidal, as is the case in *A. brasiliana* Rang. This finding will hopefully stimulate reconsideration of the visual capabilities of this species.

In addition to these general shape patterns, the lenses of some gastropods exhibit unique shape variants. The opisthobranch *Navanax* (= *Aglaia*) *inermis* (Cooper) possesses a distinctly bilobed lens, the function of which is

unknown (Eskin and Harcombe, 1977). In the eye of *Turbo castanea* Gmelin, a portion of the lens protrudes through the aperture, and this protrusion of the lens has a shorter radius of curvature than the main body of the lens located within the intraocular space. Because the focal length of a curved refractive surface is directly proportional to its radius of curvature, the protruding portion of lens causes the entire *T. castanea* lens to have a shorter focal length than it would otherwise have, making focus of light on the retina more likely. A wider field of view should also result from this 'fish-eye' lens, but this is probably less significant for *T. castanea*. Similar lens protrusions were described for *Turbo creniferus* Kiem. and *Nerita polita* Linné (Hilger, 1885), and *Neritina reclinata* (Say) also possesses a distinct lens protrusion.

Gastropod lenses vary considerably in hardness. The literature contains numerous pictures of shattered lenses, and it appears that hard lenses are more common than soft lenses in gastropods. However, exact description of lens hardness is difficult, and besides apparent lens hardness (as indicated by degree of shattering when sectioned) seems to be influenced somewhat by the fixative and embedding medium used when processing eyes for histological examination (Hamilton *et al.*, 1983). Hardness is generally correlated with refractive index, a physical property directly relevant to vision. Authors have frequently noted a concentric pattern of stain uptake by gastropod lenses (e.g. Newell, 1965; Jacklet and Colquhoun, 1983; Gibson, 1984), and have inferred from this that such lenses vary concentrically in composition, and presumably refractive index. Gibson (1984) reported that polyhedral subunits, apparently composed of protein, were packed more densely towards the lens center in *Ilyanassa obsoleta* (Say).

Refractive index patterns or gradients within lenses or other structures can be measured exactly from frozen sections using differential interference microscopy. Land has successfully employed this technique with various invertebrates (e.g. Land and Burton, 1979), although data for the lenses of gastropods have not previously been published. In this technique, the distance that an interference fringe is displaced at any given point depends upon the refractive index at that point, as well as light wavelength and specimen thickness, both of which can be controlled.

Preliminary data for the marsh periwinkle, *Littoraria irrorata* (Say), reveal a distinct refractive index gradient within its spherical lens, which is only 170 μm in diameter (Fig. 3). [Reid (1986) moved this species from the genus *Littorina*.] This gradient closely matches the theoretical gradient required for complete correction of spherical aberration (the curve in Fig. 3; based on Fletcher *et al.*, 1954), a major source of potential image degradation. Preliminary data obtained for the ellipsoidal *Aplysia brasiliana* lens also indicate a refractive index gradient, ranging from about 1.40 at the periphery to about 1.51 at the core. The *Turbo castanea* lens varies in

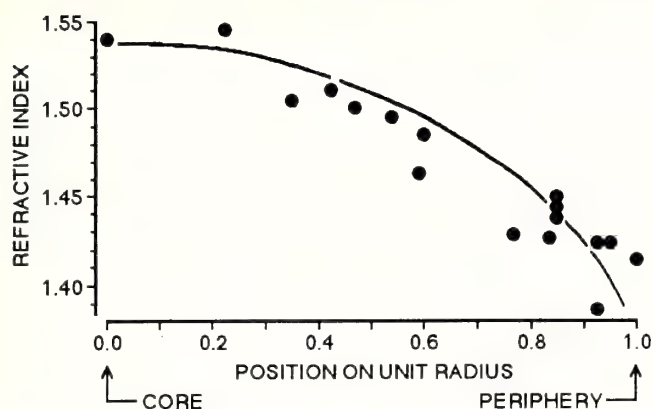


Fig. 3. Refractive index values at different points in the 170 μm diameter spherical lens ($f/r=2.71$) of the marsh periwinkle, *Littoraria irrorata*, as measured using differential interference microscopy. The curve indicates the refractive index gradient required for perfect correction of spherical aberration in a spherical lens ($f/r=2.70$) surrounded by a medium whose refractive index is 1.365, which is the average value of cornea and vitreous material.

refractive index from 1.40 at the cornea to 1.45 near the lens center, so even soft lenses can have refractive index gradients. The optical significance of a refractive index gradient is unclear for species with relatively soft lenses, and lenses with non-spherical surfaces. Substantial spherical aberration could exist in such eyes, or spherical aberration could be eliminated if corneal surfaces are parabolic, instead of spherical. In addition to reducing spherical aberration, refractive index gradients also produce a shorter-than-expected focal length, a result which could be as or more important in some species.

Gastropod eyes also vary substantially in retinal and photoreceptor properties (Table 1). Nudibranchs must be considered separately because they clearly exhibit extreme reduction of the eye. For example, Hughes (1970) found that 16 species of nudibranchs had an average of only eight (8) photoreceptors per eye. A survey of the 26 non-nudibranch species on which data are available reveals that retinal areas vary from 0.013 mm^2 (*Agriolimax reticulatus*) to 3.025 mm^2 (*Strombus luhuanus*), a factor of about 230 times. Most gastropods' photoreceptors contain few or no melanin pigment granules, and so contrast strongly with supportive cells. The photoreceptor counts or estimates reported in the literature have been obtained by various methods. My data on photoreceptor size and spacing have been obtained from sections tangential to the inner retinal surface. As shown in Table 1, adjacent photoreceptors are separated by distances varying from 2.7 μm (*Turbo castanea*) to 23.8 μm (*Strophocheilus* sp.). Photoreceptor abundances per eye vary from 40 (*Agriolimax reticulatus*) to 97,020 (*Strombus luhuanus*), a factor of about 2,500 times. These photoreceptor abundance values should be viewed only as estimates because at least some gastropods possess two or more receptor types, and these may not be easily distinguished at the light microscope level. Also, photoreceptor densities are known

to differ in different regions of the retina in *Littoraria irrorata* (Hamilton *et al.*, 1983) and *Aplysia californica* (Herman and Strumwasser, 1984).

Variation in lens or retinal properties can even be substantial within a family or genus. The greatest variation encountered thus far appears to be among the littorinids. When compared with *Littorina littorea* (Linné), *Littoraria irrorata* has twice the retinal area and a three times shorter receptor separation distance, which results in almost 16 times more receptors per eye (Table 1). *L. irrorata* is active in air, while *L. littorea* appears principally active when submerged in water. That difference in behavioral ecology is probably associated with the substantial difference in eye structure, because the degree to which light is refracted at the cornea depends greatly upon the refractive index of the surrounding medium. *Tectarius muricatus* (Linné), another littorinid, is active in air. Although it has a retinal area intermediate between the other two littorinids, it has a receptor separation distance and total receptor abundance that are much more similar to the littorinid active in air, *L. irrorata*. These similarities in retinal design are related presumably to the higher light levels present in air. Unfortunately, the data available for the eye of *Littorina scutulata* Gould (Mayes and Hermans, 1973) do not allow evaluating the eye as an optical system.

Variation in eye structure in littorinids can be explained by differences in behavioral ecology, but the situation is less clear in other cases of variation between closely related gastropods. Within the Strombidae, *Strombus luhuanus* and *Pterocera* (= *Lambis*) *lambis* have similarly sized eyes, but *S. luhuanus* has twice as closely spaced receptors and hence four times more receptors than *P. lambis*. The receptor which Prince (1955) illustrated for *P. lambis* is 6 μm in diameter; however, he indicated that the average receptor diameter is 11 μm , and his estimate of total receptor density ("something approaching" 10,000/ mm^2) generally agrees with the estimate in Table 1, which is based on a receptor separation distance of 12 μm . Both strombid species are active in shallow water. *S. luhuanus* is about half the size and travels almost nine times more slowly than *Pterocera* (= *Lambis*) *lambis* (Berg, 1974), yet the *S. luhuanus* eye seems capable of resolving greater detail. Berg (1974), who studied ten strombids, noted specifically that *S. luhuanus* seemed "to be able to sense the position" of a predatory cone snail, but he did not speculate on the sensory modality involved. No obvious differences are apparent in eye structure between the two *Aplysia* species listed, beyond the disagreement mentioned earlier about shape of intraocular space and lens. This is somewhat surprising because the species seem to exhibit basic behavioral differences. *A. brasiliiana* is an excellent swimmer and is active principally at night. In contrast, *A. californica* apparently does not swim at all, and is diurnally active in the lab and field; whether it may also be nocturnally active under

natural conditions remains unknown (Hamilton, 1986; Leonard and Lukowiak, 1986).

Vision in various species can most accurately be compared, not by any of the numbers in Table 1, but rather by knowing the resolving power of the eye. Several factors can influence resolution, but one useful estimate of resolution is the angular separation of adjacent receptors relative to the 'optical center' of the eye (the posterior or proximal nodal point). Resolution measures or estimates have been published for *Littorina littorea* (Newell, 1965), *Littoraria irrorata* (Hamilton *et al.*, 1983), *Strombus luhuanus* (Land, 1984), *Heliotis discus* (Land, 1981), *Biomphalaria glabrata* (Say) (Schall and Baptista, 1989), and *Strophocheilus* sp. (Oswaldo-Cruz and Bernardes, 1982), and reasonable estimates can be computed for a few other gastropods. These resolution values are given in figure 4, along with comparative data for selected arthropods and vertebrates from Kirschfeld (1976). Clearly the resolving powers possessed by gastropod eyes exhibit a wide range, and they overlap considerably with the resolving powers of arthropod and vertebrate eyes.

As Audesirk and Audesirk (1985) suggests, the assumption that vision plays only a minor role in gastropod behavior has had an inhibitory influence on careful studies of visually-mediated behavior in this group. It is commonly believed that the structurally-simple eyes of gastropods only mediate simple phototaxis or skototaxis, orientation toward or away from light or dark areas, respectively. However, critical experiments have rarely been done that could allow discrimination between true phototaxis or skototaxis, and form vision, however crude or simple. Hopefully, the existing collection of anecdotes and

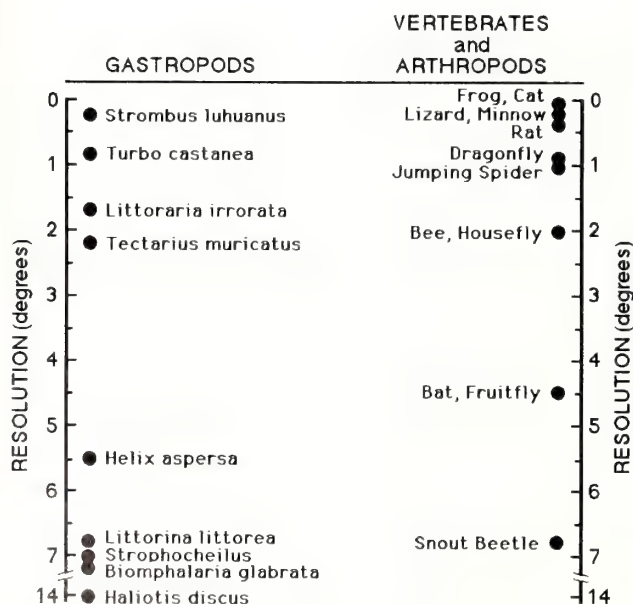


Fig. 4. Measures and estimates of anatomical resolution for nine species of gastropod, with comparative data for selected vertebrates and arthropods.

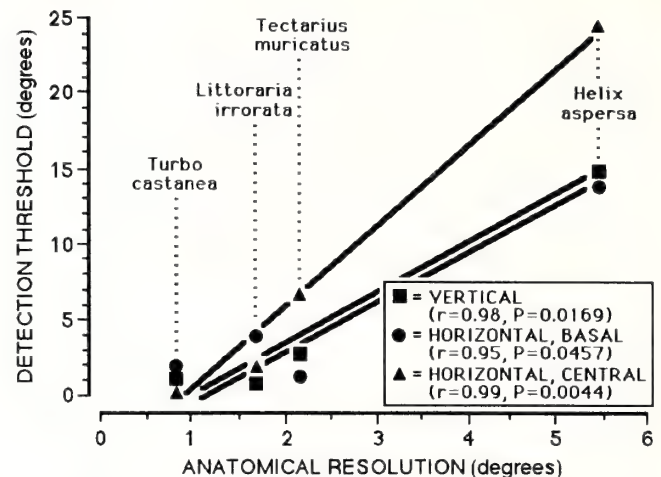


Fig. 5. Measures and estimates of anatomical resolution, and behavioral 'detection' thresholds for oriented responses to three types of targets (vertical stripe, centrally-positioned horizontal stripe, basally-positioned horizontal stripe), for four species of gastropod.

weakly founded assertions about gastropod vision will eventually be replaced with carefully obtained data delineating their visual capabilities.

Standardized behavioral measures of visual detection have been obtained for four of the gastropod species discussed here (Hamilton and Winter, 1982, 1984), and these compare well with the previously mentioned anatomical resolution measures and estimates (Fig. 5). Also, it is clear that at least *Littoraria irrorata* can distinguish details of an object's orientation; it preferentially moves toward a vertical black stripe on a white background when presented with horizontal or diagonal stripes having equivalent width and contrast (Hamilton and Winter, 1982). Because the *L. irrorata* eye is not qualitatively different from that of many other gastropods, it could well be that other species can distinguish such visual detail too. Both *Tectarius muricatus* and *Turbo castanea* show some ability to discriminate target orientation (Hamilton and Winter, 1984). An unblocked view of the sky is required for *Aplysia brasiliana* to maintain its swimming direction, which suggests sensitivity to complex visual cues (Hamilton and Russell, 1982a).

In summary, the assortment of optical tricks encountered in the eyes of various vertebrates, including fish eye lenses and lenses with refractive index gradients, are also found among gastropods. This high degree of variation in gastropod eyes should not be viewed as counter-intuitive. The fishes, for example, are less diverse in habitat and general morphology than the gastropods, yet their eyes exhibit an extensive range of adaptations correlated with habitat and behavioral strategy (Lythgoe, 1980; Fernald, 1988). As Land (1981) has noted, there is no clear break within the range of resolving powers exhibited by the eyes of animals. There really is no such thing as an image forming eye as distinct from

a non-image forming eye. There are only degrees of need for visual detail among animals, and degrees of image quality provided by eyes. For gastropods and other invertebrates, assumptions about vision, based on vertebrocentric biases, need to be replaced by more hard data and a genuine comparative perspective.

OPISTHOBRANCH RHINOPHORES

This is one of the most obvious cases of unappreciated variation in molluscan sensory biology, and a classic example of why biologists should avoid assigning names to structures that are based on assumed functions. The rhinophores are a pair of tentacles located near the eyes on the dorsal surface of the head, and hence in the same location as the pair of tentacles termed the 'cephalic tentacles' in prosobranch gastropods. The name 'rhinophore' literally means bearer of the nose or nasal sense. This name appears to have been coined by Bergh (1879), who worked principally with the predatory nudibranchs, a group in which the rhinophores do seem involved in distance chemoreception in many species. MacFarland's (1966) treatise on opisthobranchs beautifully illustrates the structural diversity of nudibranch rhinophores. In many species, rhinophoral sheaths are present, as well as numerous lamellae projecting laterally from a central axis. The greatly increased surface area provided by the lamellae is itself suggestive that nudibranch rhinophores have a chemosensory function in this carnivorous group.

However, if one takes a more comparative approach, and looks at other opisthobranch taxa, it is clear that other feeding strategies exist, and that the so-called rhinophores can have a variety of different structural features and sensory functions. In the herbivorous *Aplysia*, for example, each rhinophore has a simple gross structure, with no basal sheath and no lamellae. A pigmented groove on the distal half of the rhinophore receives most of the innervation (Fig. 6; from Ronan, 1990). Despite this much simpler gross structure, aplysiid rhinophores have been implicated in chemoreception, mechanoreception (touch, waves, currents) and even photoreception (Frings and Frings, 1965; Jahan-Parwar, 1972; Audesirk, 1975; Chase, 1979; Jacklet, 1980; Hamilton and Russell, 1982b). It could be that variation exists in rhinophore sensory function, even among *Aplysia* species, since substantial variation exists in morphology, activity rhythms and swimming behavior among aplysiids (Eales, 1960; Hamilton, 1986).

Inadequate appreciation for the variation in rhinophore morphology, feeding strategy and key behavioral traits between nudibranchs and other opisthobranchs has led to the incorrect assumption that the sensory functions associated with the rhinophores of a predatory group (the nudibranchs) automatically apply to other opisthobranch groups. A general under-appreciation for diversity within opisthobranchs could

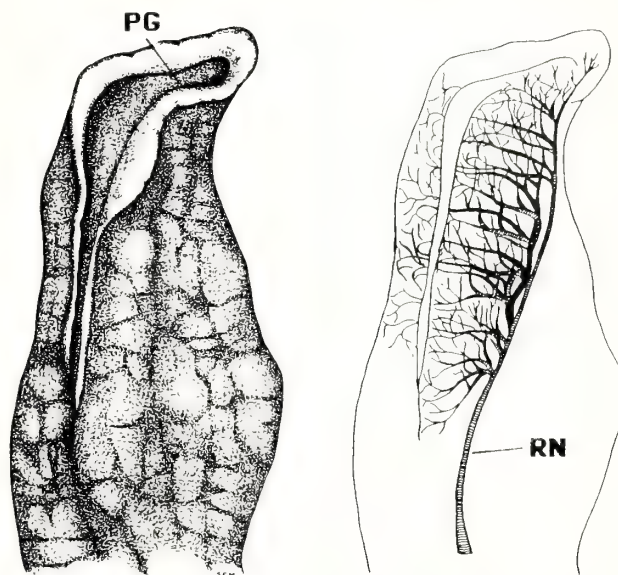


Fig. 6. External view showing the pigmented groove (PG) of the *Aplysia brasiliana* rhinophore (left), and internal view showing the rhinophoral nerve (RN) and its innervation pattern (right), based on analysis of serial sections (from Ronan, 1990, with permission). A fully extended rhinophore is 15-20 mm long in adults.

be partly responsible for this problem, but the literal meaning of 'rhinophore' is probably the primary factor. Beginning ethologists are taught the importance of selecting names for the discrete behaviors included in an ethogram based on spatial and temporal features of the movement pattern involved, rather than on the presumed adaptive function of the behavior. For example, the cyclic lateral movement of the siphon which appears when various neogastropods become alerted to a prey's proximity would be named something like 'siphon waving' rather than 'odor searching'. Anatomists are presumably taught some similar rule, and certainly the 'issue' of opisthobranch rhinophores would be less confused today if such a standard had been followed in the 1800's. The confusion that the term rhinophore has caused over the years suggests that we could be better off just referring to these opisthobranch structures as cephalic tentacles, as we do for the seemingly homologous paired tentacles of prosobranch gastropods. Admittedly, the term 'tentacle' suggests a tactile sensory function, but mechanoreception seems a general property of virtually all such structures, regardless of what additional sensory capability they can exhibit.

SCALLOP EYES

Scallops (family Pectinidae) are swimming bivalve molluscs which possess about 60-100 eyes distributed along the mantle edges. The detailed studies of Land (1965) on *Pecten maximus* (Linne) revealed the presence of a unique double-layered retina in each eye, and an equally unique

optical system dependent upon a reflector at the rear of the eye, whose surface is described as spherical. Importantly, Land's analyses show little or no space between the rear of the lens and the retina, or between the retina and the reflective argentea. This is in contrast to Dakin's (1928) earlier study of *Pecten maximus*, which reported a space between the lens and retina about 20% of the eye's length. Butcher (1930) found no space between the lens and retina of *Pecten* (= *Argopecten*) *gibbus* (Linneé) but he found a space occupying about 40% of the eye's length between the retina and argentea of this species. In a preliminary study of the eye of *Argopecten irradians* (Lamarck), Wooters (1989) found a space having a similar size and location to that reported by Butcher, and an argentea which appeared more parabolic than spherical. Finally, considerable variation exists in the sizes of the eyes possessed by individuals in at least some scallop species. This variation could be due to differences in developmental or regenerative growth, but this appears unstudied with the exception of Butcher's (1930) work on *Pecten* (= *Argopecten*) *gibbus*. Perhaps the apparent interspecific differences in scallop eye structure are due to different investigators describing eyes at different growth stages in different species.

As with gastropod molluscs, any genuine interspecific differences in scallop eye structure could also be associated with differences in behavior or ecology. *Argopecten irradians* are found in water less than 4 m deep, particularly in beds of the seagrass *Thalassia*, whose blades reach as high as 0.4 m above the bottom. In contrast, *A. gibbus* and *Pecten maximus* are both found in water deeper than 10 m, typically on substrata consisting of sand and shell fragments. Hence, these three scallops differ in the amount of biologically relevant visual detail in their environments, as well as in water depth and associated light characteristics. How these habitat differences or behavioral traits could be associated with differences in eye structure is unknown. Wooters (1969) found that *A. irradians* orient visually toward grassbeds when released in sand patches adjacent to grassbeds, but little is known of the behavioral role of vision in other scallops.

In summary, detailed comparative studies need to be completed to determine exactly what ontogenetic and interspecific variation exists in scallop eye structure and optical properties. Although the eyes of the various scallop species seem generally similar, the available information on eye structure suggests that some interspecific differences could exist in optical properties, especially as regards focal point position and the degree of spherical aberration. Insufficient attention to interspecific variation can easily lead to confusion, and possibly even errors in interpretation. For example, McReynolds and Gorman (1970) studied the electrophysiological properties of the eye of *Pecten* (= *Argopecten*) *irradians*, but included a "*Pecten* eye" diagram based on Dakin's (1928) illustration of the eye of *Pecten maximus*. It

seems prudent to exercise caution in extrapolating structural, physiological and behavioral results across scallop species until detailed comparative studies are conducted.

CONCLUSION

This review of gastropod vision, opisthobranch rhinophores and scallop eyes demonstrates that there exists considerable variation in sensory system design and capabilities among molluscs, even within families or genera. In the case of gastropod eyes, considerable progress has been made in documenting substantial variation, and the problem seems that the observed variation is underappreciated. In the case of scallop eyes, interspecific variation has barely been documented, yet preliminary results encourage detailed comparative study in the future. The case of opisthobranch rhinophores seems somewhat intermediate; appreciation that the function implied by the name rhinophore might not apply to many opisthobranchs has existed at least since Arey (1918), yet little progress has been made toward obtaining a satisfactory comparative view of the behavioral and ecological correlates of the variations in structure found in this group.

Additional evidence of substantial interspecific variation will likely be obtained eventually for other sense organs, such as the statocyst and osphradium. Statocyst structure has been examined for a modest number of molluscs (Budelmann, 1988), but the possible behavioral-ecological correlates of observed structural diversity have hardly been explored. The literature contains some interesting and apparently paradoxical cases which beg for analysis. For example, the benthic gastropod *Pomacea paludosa* (Say) possesses about 3,000 sensory cells in its statocyst, while a mere 13 cells are present in the statocyst of *Aplysia limacina* de Blainville (= *A. fasciata* Poiret), which is one of the many aplysiids which has been reported to swim as well as crawl on the bottom (Dijkgraaf and Hessels, 1969; Stahlschmidt and Wolff, 1972). Analysis of osphradial variation and its basis across molluscs is complicated by the fact that the name osphradium has sometimes been assigned to structures that are clearly not homologous.

The presence of this substantial interspecific variation emphasizes the necessity of communicating about particular organisms and sense organs more carefully and, where possible, more exactly. The limits and bases of variation would be more easily understood if more authors would include specific information about the sizes of the actual animals they studied, as well as the relationship between sense organ dimensions and body size in the species. Zinke's (1978) study of the eye of *Succinea* includes detailed coverage of ontogenetic variation and, unfortunately, it is unusual in doing so. Failure to consider intraspecific or ontogenetic variation can lead to generalizations just as premature and flawed as those based on too few or atypical species. Autrum (1979) pointed out that it is clearly a bad habit to speak just of "the

fly'' in scientific writings. Likewise, it can be a bad habit to speak just of ''the littorinid'', ''the rhinophore'' or ''the scallop'', depending on what species, system or capability is being discussed.

Finally, as more comparative data become available, it should become possible to discard some of the less informative terms in general use for sensory structures. For example, there have existed in the invertebrate literature several terms for structurally simple eyes. 'Pigment cup eyes' and 'pinhole eyes', which both lack lenses, have widely accepted definitions based on structural features (see Land, 1981). However, 'eyespot' and 'ocellus' both mean ''a very small simple eye formed in invertebrates.'' As has so often been the case in invertebrate zoology, the emphasis seems to have been on establishing the existence of a difference as compared to the vertebrate eye, rather than on recognizing the various invertebrate eyes as being distinct structures worthy of independent study and understanding.

As a relatively new field, many gaps exist in our knowledge of molluscan behavior and sensory biology. A clearer understanding would be provided about sensory capabilities within groups if more species were examined, and this would also allow more thorough investigation of the basis of existing variation across species. However, more effort should also be devoted to completing the data sets for species about which some information is already available. In many cases, only data on structure of sense organs or sensory tissues are available from which to draw inferences about function; obviously the availability of data from electrophysiological and behavioral studies would be desirable in such instances. For example, scallop eye structure and electrophysiological responses have received some study, but there is little understanding of how well they can see or what adaptive value vision has in nature. A reasonably complete range of data are available for only a few cases, mostly involving species that serve as model systems for neurobiologists. Thus, future researchers in molluscan sensory biology should be encouraged to flesh out existing stories, as well as to expand our understanding of variability within the group by examining as-yet-unstudied species.

ACKNOWLEDGMENTS

I thank W. Jones for assistance in computing partial surface areas of ellipsoids, S. Miller for drawing figure 6, and C. N. D'Asaro and J. S. Wooters for assistance and critical comments. Portions of this research were supported by NSF grants BNS79-16358 and BNS83-08186, and by an award from UWF's research committee. This is contribution 91-4 of the Institute for Coastal and Estuarine Research.

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Date of manuscript acceptance: 16 April 1991

Research Note

Sperm storage and evidence for multiple insemination in a natural population of the freshwater snail, *Physa*

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Abstract. Although a number of estimates are available regarding the capacity of freshwater pulmonates to store sperm after laboratory mating, few such data are available for wild-collected snails, where the recency of mating is necessarily unknown. We collected 35 *Physa heterostropha pomilia* (Conrad) from a local population and held each in isolation for 60 days, rearing all egg masses. We then used protein electrophoresis to determine the LAP genotype of each parent and a sample of its offspring. Five of the parental snails (total 60 day fecundities from 300 - 600 progeny) were found to be homozygous at the LAP locus yet producing approximately 50% heterozygous offspring. In 4 of these 5 cases, no significant difference was detected in offspring genotype frequencies over 60 days, suggesting both that mating has generally been recent and that reservoirs of stored sperm are, as a rule, large. In the fifth case, the frequency of heterozygous offspring increased significantly, suggesting multiple insemination. Multiple insemination and sperm storage have obvious adaptive significance to colonizing species such as freshwater pulmonates.

The capability to store sperm, especially sperm contributed by multiple partners, has the potential to lessen the severity of genetic drift by increasing the effective population size represented by a small number of survivors or colonizers. And the capacity of an individual pulmonate snail to store sperm can be prodigious. In *Bulinus*, laboratory mating has been reported to provide enough sperm to fertilize from 1000 - 2000 eggs up to 4400 eggs, depending particularly upon the reproductive condition of the snail acting as female (Rudolph, 1983; Rudolph and Bailey, 1985). In *Biomphalaria*, longevity of stored sperm has been variously estimated as 25 - 68 days (Paraense, 1955), 42 days (Richards, 1973), and more than 100 days (Monteiro *et al.*, 1984). Vianey-Liaud *et al.* (1987) reported a mean of about 50 days with a range from 3 days to 127. Cain (1956) reported some exogenously fertilized egg production by *Lymnaea stagnalis* Say up to 116 days after isolation. All these studies have involved lab crosses with pigment variants (usually albinism) as a genetic marker. Rollinson and Wright (1984) used isozyme markers to demonstrate sperm storage up to 70 days after laboratory mating of Mauritian *Bulinus*.

The effect of a delay in oviposition by the mother (as by a severe winter or desiccation on a birds foot, for example) has been investigated by Rudolph and Bailey (1985). Apparently *Bulinus* can store viable exogenous sperm through a minimum of seven weeks of starvation, eight weeks of low

temperature (10 - 15°C) or four weeks of desiccation.

Rudolph and Bailey (1985) also reported some fairly strong evidence that *Bulinus* can store sperm from more than a single male simultaneously. Although short-lived copulatory plugs have been described in several pulmonate species, behavioral observations nevertheless suggest that multiple insemination could be common in laboratory situations (Rudolph, 1979a, b; van Duivenboden and ter Maat, 1988). Duncan (1959) reported that he had not observed reciprocal copulation in either *Physa fontinalis* (L.) or *P. acuta* (Drap.). But we have observed both reciprocal copulation and multiple mating behavior in our laboratory populations of *Physa*, without genetic markers for confirmation.

Using isozyme markers, Mulvey and Vrijenhoek (1981) found strong evidence of multiple paternity in clutches of eggs laid by isolated wild-caught *Biomphalaria*. It could not be determined, however, if the sperm used to fertilize these eggs came from two different exogenous sources, or from a combination of one outcross and selfing. It does in fact seem that fertilization can proceed with endogenous and exogenous sperm simultaneously, at least in some situations (Paraense, 1955; Monteiro *et al.*, 1984; Rollinson, 1986). But multiple insemination has been conclusively documented by Rollinson *et al.* (1989), using stocks of *Bulinus cernicus* (Morelet) homozygous for three different alleles at the Gpi locus.

As important as they are, data from lab crosses such as these do not directly address the likelihood of genetic drift after a population crash or founder event. A second set of

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variables is involved: how recently will an arbitrarily chosen pulmonate snail have mated? On the average, how healthy and fecund will it be? Rollinson (1986) has performed experiments bearing on this question using several species of African *Bulinus*. He isolated individual wild-caught snails and collected and reared their eggs up to 60 days. He then examined isozyme phenotype of both parent and offspring at four polymorphic loci. The large majority of these parents did lay demonstrably outcrossed eggs, apparently using stored sperm. Stored sperm was used for at least 41-44 days. Intriguingly, Rollinson found one *Bulinus scalaris* (Dunker) clearly producing offspring from two different fathers.

The great majority of all observations to date have involved lab matings of the tropical planorbids *Bulinus* and *Biomphalaria*. So to test the generality of the sperm storage phenomenon in natural populations of temperate pulmonates, we performed an analysis similar to Rollinson's on a local population of *Physa*.

METHODS

Physa heterostropha pomilia (Conrad) is a widespread and variable species, found throughout eastern North America (Wurtz, 1949; Burch and Tottenham, 1980). Recently Te (1978, 1980) has suggested that this taxon, along with the majority of North American species, be separated into the resurrected genus *Physella*. But we agree with Taylor (1988) that the distinction between *Physella* and *Physa* (s.s.) is more proper at the rank of subgenus.

We collected *Physa heterostropha pomilia* from a pond at Charles Towne Landing, a state park within the city limits of Charleston, South Carolina. The snails did not appear to be common on this nor on any subsequent trip, and we felt it possible that individual encounters and matings could be infrequent in this population. Voucher specimens have been deposited in the Academy of Natural Sciences of Philadelphia.

For an initial survey of polymorphism, we homogenized 62 snails in a 7% sucrose solution, buffered at pH 7.4 with 0.05 M tris (hydroxymethyl) aminomethane and H_3PO_4 , to which xylene cyanole had been added as marker. Samples were centrifuged and horizontal starch gel electrophoreses was performed on the supernatant using standard techniques (Dillon and Davis, 1980; Dillon, 1982, 1985). The 14% starch gels were made of 3 parts Sigma starch: 1 part Electrostarch and AP6 buffer, diluted 19:1. AP6 buffer is 0.04 M citric acid (monohydrate) adjusted to pH 6 with N-(3-aminopropyl) morpholine (Clayton and Tretiak, 1972). Gels were run for approximately 4.5 hr at 40 volts under refrigeration. They were then sliced and stained for leucine aminopeptidase (LAP) using the recipe of Shaw and Prasad (1969).

Two isozymes were found to be segregating in a fashion consistent with Hardy Weinberg expectation in the Charles

Towne Landing population, at gene frequencies of 0.58 and 0.42. We have designated the more common isozyme "LAP 100", and the isozyme migrating 3 mm faster in our gel conditions "LAP 103".

We returned to Charles Towne Landing in May, 1989, and collected 35 adult *Physa*, placing each in a separate 10 oz plastic cup of pond water with a plastic petri dish cover. We fed them commercial Tetra-Min "Conditioning" food for plant-eating fish (Jennings *et al.*, 1970), and changed the water with fresh, aerated pond water periodically. Each parent was checked daily and transferred to a new cup when an egg mass was produced, in a fashion similar to that of Rollinson (1986). Water was changed once a week for the adults (along with newly laid egg masses) and once every other week for the juvenile snails when they had grown to a size at which this could be done safely.

The experiment was terminated in July (after 60 days), by which time 29 of the parents had reproduced, some prolifically and others much less so. We then determined the genotypes of each parent and a sample of early laid offspring at the LAP locus. Of the 15 LAP homozygotes identified, four were producing all homozygous progeny and the remainder were producing high frequencies of heterozygotes, obviously using exogenous sperm. We did not find any case where a homozygous parent was producing entirely heterozygous offspring, as could be expected from a single outcross to the opposite homozygote. But we selected for further study the five largest sibships from the 11 including heterozygotes. We then compared the frequency of heterozygotes in 20 to 30 offspring from the earliest egg masses in these sibships to a similar sample from the last laid sibships.

RESULTS

Data on the fecundity of these five snails (A through E) during the 60 day study period are presented in figure 1. The figure shows the cumulative number of juveniles surviving to countable size. It can be seen that all parents continued to lay eggs throughout the entire period, although the rate slowed, especially after day 45. Total viable egg masses ranged from 15 to 19, and the total countable offspring ranged from about 300 to 600.

Table 1 shows the frequency of heterozygotes in 20 to 30 offspring of the first laid sibships and the frequency of heterozygotes in similar sized samples from the last laid sibships. Genotype frequencies were not significantly different from 1:1 (chi square, Yates corrected) in any of the five groups of first laid offspring. This is consistent with Mendelian expectation if each mother had mated with a single heterozygous father.

Table 1 also shows that in four of the five cases, there was no significant difference between the genotype frequencies in the first laid sibships and the last laid sibships.

Table 1. LAP genotypes among the offspring of five parent *Physa*, first laid sibships compared to last laid sibships.

Parent	LAP Genotype	Day Number of Oviposition	Number of Homozygotes	Number of 100/103 Heterozygotes	chi-square
A	103/103	4,9	20	15	1.44
		58,60	16	5	
B	100/100	7,11	16	17	6.63**
		51,52,53,56	2	18	
C	100/100	2,8	17	13	0.36
		56,57,58	11	7	
D	103/103	2	19	15	1.04
		44,51,56	9	14	
E	103/103	7	13	17	0.13
		51,52,56	12	11	

**P < 0.01

The one significant value of chi square (contingency test, Yates corrected) is shown in the offspring of parent B, where there was an unexpected excess of heterozygotes among the offspring laid in the final days of the experiment.

DISCUSSION

We do not know whether the total fecundities reflected in figure 1 overestimate or underestimate production by a single *Physa* founding a new population in the wild. On the one hand, we attempted to provide food in excess and protected the juveniles from predation. But competition among juveniles could have been an important factor in our plastic cups, especially on occasions when as many as three egg masses were laid overnight.

Although it can be seen that all parents continued to lay eggs for the entire 60 day period, there was a reduction in the number of viable embryos per egg mass through time. For example, the last three egg masses laid by snail E, on days 57, 58, and 60, contained no viable embryos at all. Qualitatively these results are similar to those obtained by

Duncan (1959) with English *Physa fontinalis*, although *P. heterostrophia pomilia* fecundities seem to be higher. As previous work suggests a gradual shift from exogenous to endogenous sperm (Paraense, 1955; Cain, 1956), we initially interpreted this observation as evidence of depleted sperm stores. But Table 1 shows that after 60 days and as many as 600 offspring, none of these individuals seems to have exhausted its reservoir of stored sperm. In retrospect, our decision to terminate the experiment was premature.

The excess of heterozygotes observed among the progeny of parent B is not easily explained as the result of a single pair mating. If the offspring were fathered by a single heterozygous individual, one would expect a 1:1 ratio of homozygous to heterozygous progeny. This is indeed the ratio observed in all early sibships, laid on days 2 through 11 (Table 1). But the sample of snail B progeny from days 51, 52, 53, and 56 contained 18 heterozygotes and only 2 homozygotes, very significantly different from 1:1. Even combining all 53 progeny examined from parent B, one still obtains a significant excess of heterozygotes (goodness-of-fit chi square = 4.79). Nor can these results be explained by a single cross to a homozygous father, as no homozygous progeny would have been expected at all. To argue that self fertilization played any role, one would need to postulate that parent B was at least partly self fertilizing to start but increasingly shifted to exogenous sperm as the days in isolation passed, quite counter to all previous observations on other pulmonates.

By far the most likely explanation for the results from parent B is multiple insemination by both a heterozygote and a homozygote for the opposite allele. The first eggs seem to have been fertilized by sperm from the former, and the last eggs by the latter, in a fashion similar to that inferred for *Biomphalaria* (Mulvey and Vrijenhoek, 1981) and well documented for *Bulinus* (Rudolph and Bailey, 1985; Rollinson, 1986; Rollinson *et al.*, 1989). Clearly more work is called for, possibly using multiple loci as markers for different parents. But if multiple insemination is in fact

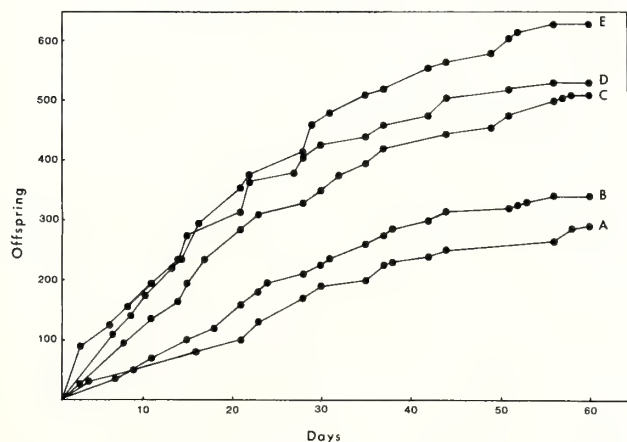


Fig. 1. Cumulative 60-day fecundity (offspring surviving to countable size) of the five *Physa* examined for sperm storage.

widespread, individual pulmonate snails surviving colonization events, hard winters or severe storms could potentially represent a great deal of genetic variation indeed.

Given the apparently large capacity *Physa heterostroph* displays for sperm storage, it would be interesting to see how frequently an average snail mates, and in what capacity. The great majority of freshwater pulmonate species show either simultaneous development of both reproductive tracts, or are slightly protandric (Russell-Hunter and McMahon, 1976; Rudolph, 1983). The male organs develop before the female organs in *P. fontinalis*, although environmental conditions in the wild do not in general favor mating until both organ systems are mature (Duncan, 1959). It is not clear whether fully mature pulmonates of any sort prefer to mate as a certain sex, whether a snail's sexual role can change with size or sperm stores, or how often snails switch roles in single encounters. Multiple insemination introduces questions of sperm competition and sperm "sharing" (Monteiro *et al.*, 1984; Vianey-Liaud *et al.*, 1987).

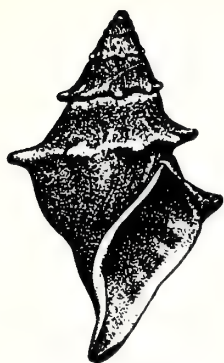
ACKNOWLEDGMENTS

We thank Charles Towne Landing State Park and Mike Dorn for access to the *Physa* population and Dr. Margaret Mulvey for her advice on pulmonate culture.

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Date of manuscript acceptance: 10 September 1990



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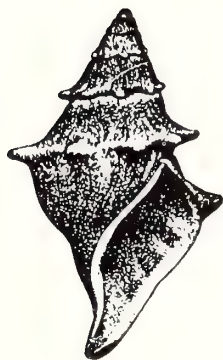
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VOLUME 9

NUMBER 2

Biannual Journal of the American Malacological Union

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The Bivalvia: Future directions for research

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The theme of this paper and attendant symposium is "Future Research Directions for the Bivalvia". This paper presents a personal view about a class of animals for which we share a common interest and enthusiasm.

My involvement with the Bivalvia goes back many years when I first began researches upon *Dreissena polymorpha* (Pallas), then and now a continuing problem in the exploitation of fresh waters in Europe. I left *Dreissena* in 1969, when I completed my Ph.D. and, at the same time, left Great Britain to take up a teaching post in Hong Kong. I believed I had left *Dreissena* for ever. Just this year, however, I have been asked to write the introductory chapter for a book on *Dreissena*, the stimulus for publishing such a volume being the introduction of this species into North America via the Great Lakes and the economic consequences of the introduction that are already being felt. I will return to the general topic of introductions later, but it is worth noting, in passing, that the Bivalvia, despite their sedentary habits, do have a tendency to travel to the most unlikely places.

I was a student of Professor R. D. Purchon who was, in turn, a student of the late Sir Maurice Yonge, arguably the father of modern research upon the Recent Bivalvia. I first met Maurice in 1967 when he visited the University of London to talk to the student Biology Society of which I was Secretary. He and I, of course, discussed *Dreissena* for he was working on it too and so a friendship was founded that lasted for 20 years until his death in 1986. I was responsible for organizing a symposium on the Bivalvia in his honour but which was, in the event, in his memory, at the IX Malacological Congress in Edinburgh in 1986. He died just a few months before the meeting was convened. The Proceedings of that Symposium were subsequently published in 1990 (Morton, 1990a).

Maurice and his students of the Bivalvia, for example, G. Owen, J. A. Allen, T. H. J. Gilmour, R. G. B. Reid, A. D. Ansell and R. D. Purchon, have had a powerful impact upon not only our understanding of the Recent representatives of this class, but also upon the Mollusca and Zoology in general for much of this century, notably in Great Britain. Some of these students have emigrated to Canada

and the U.S.A., for example, where their influence, and thus Maurice's, persists. North America too has fostered endemically students of the Bivalvia through such eminent scientists as K. M. Wilbur, R. D. Turner, S. M. Stanley, M. R. Carriker, K. J. Boss, J. Pojeta and N. D. Newell, not forgetting such legendary characters as W. H. Dall, W. R. Coe, T. C. Nelson and V. L. Loosanoff. This is not, however, going to be a discussion about famous bivalve malacologists and you have to forgive me if I have not mentioned your name or that of a mentor you think significant and comparable with those just identified. I am similarly not ignoring equally famous bivalve malacologists from, for example, Europe (V. Scarlato), Australia (B. Runnegar) and elsewhere: the only point I am trying to make is that there are rich genealogies of eminent zoologists who have made the Bivalvia their own and stamped their mark upon a group that today is close to serving as a classic model of adaptive radiation. Some of our most eminent zoologists are students of the Bivalvia. The class is of importance and interest.

With this in mind, I looked back over the last twenty-five years of *The Journal of Molluscan Studies*, so renamed after 1976 from its official status as the *Proceedings of the Malacological Society of London*, one of malacology's most prestigious societies and which, in 1993, will celebrate its centenary. I counted the number of papers published in each volume, their page length and noted which of them were concerned with the Bivalvia. The results of this simple analysis are shown in figure 1 wherein it can be seen that (A), the number of papers published annually has increased (the quantum jump in 1982 results from the initiation of the publication of Research Notes) although (B), page length, has decreased somewhat (the decline in 1982 again results from the initiation of the publication of Research Notes). Look, however, at figure 1C. The number of papers published on the Bivalvia since a heyday in 1967, when 67% of the papers were on this class, has declined progressively. So, I suspect, has the variance. In the 1960's and 1970's, volumes would contain between 25% and 45% information on the Bivalvia. In the 1980's such figures were between 10% and 25%. In 1990, the figure is approximately 10%. Extrapolating

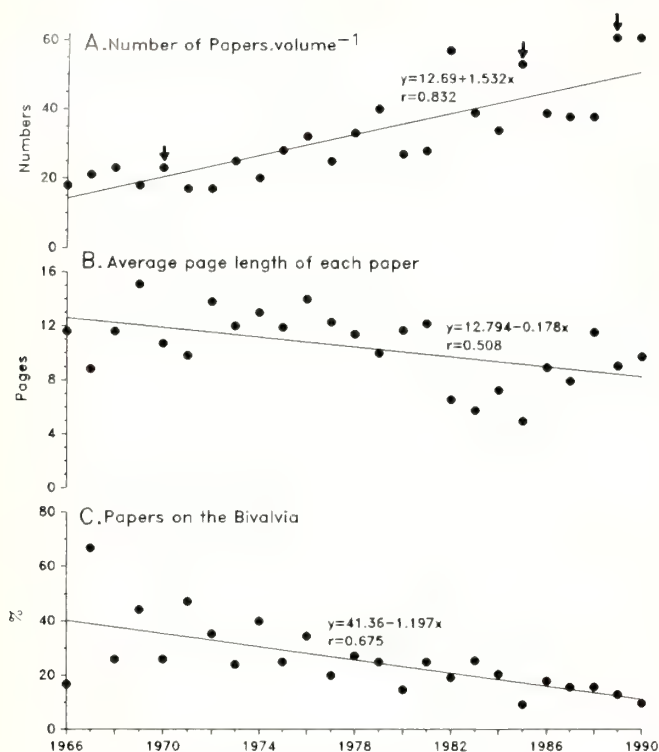


Fig. 1. A, Numbers of papers; B, average page length of each paper; C, % numbers of papers on the Bivalvia published in *Journal of Molluscan Studies* (1967-1990).

such a graph, I have concluded that *The Journal of Molluscan Studies* will cease to publish papers on the Bivalvia by about the time the parent society celebrates its centenary. I have been invited to give the plenary lecture on the Bivalvia at the Conference to celebrate the Society's centennial: it rather looks as though I will be talking to myself! The editors of the *Journal* over these years have been N. B. Eales, A. Graham and J. D. Taylor, all gastropod workers, but I am not suggesting uncharitably that they are responsible for this decline. Rather, it appears that there is a declining interest in this class of the Mollusca, at least in Great Britain.

I have also taken an American malacological journal, *Veliger*, appropriate because of its affiliation to the California Malacozoological Society, and performed the same analysis of the published papers. Here, a similar trend is apparent with regard to the numbers of papers being published (Fig. 2A) although their average length (Fig. 2B) is increasing, unlike papers in the *Journal of Molluscan Studies*. So, too, however (Fig. 2C), is the incidence of papers on the Bivalvia, again in contrast to the *Journal of Molluscan Studies*.

We thus see a fundamental difference in trends with respect to the Bivalvia in the British and American journals. I do not, however, wish to make too much of this, after all there are dozens of malacological journals and an analysis

of them all would be needed to obtain a clearer picture. I will make one point, however. The loss to North America of some of C. M. Yonge's students, for example, R. G. B. Reid, T. H. J. Gilmour, W. D. Russell-Hunter and P. V. Fankboner, and their potential to, in turn, engender students of the Bivalvia can help us to explain the decline in British studies of the Bivalvia and the increase in North American. Scientific emigration from Britain has been going on for decades and is a damning indictment of British Government policy with regard to its science and its scientists. But is there a wider trend with regard to research upon the Bivalvia?

With the help of my colleague, Professor J. Britton, I have conducted a library search of BIOSIS for the numbers of papers published on the Mollusca, Bivalvia, Gastropoda and Cephalopoda since 1969, i.e. the last 22 years. Numbers of papers published on the Mollusca have, as might be anticipated, increased from 379 in 1969 to 801 in 1990, i.e. an overall increase of 422 or 113.5% (Table 1). Numbers of papers on the Cephalopoda, Gastropoda and Bivalvia have increased by 13 (11.9%), 232 (128.9%) and 177 (135.1%), respectively. The largest overall percentage increase is, thus, upon the Bivalvia. Looking at publications upon the three classes in terms of their percentage contribution to the molluscan body of literature (Fig. 3), trends become apparent. The relative number of papers published on the Cephalopoda has declined, since 1969, by as much as ~ 50%. Research on the Gastropoda seemed to peak in about 1972, corresponding with a trough in publications on the Bivalvia.

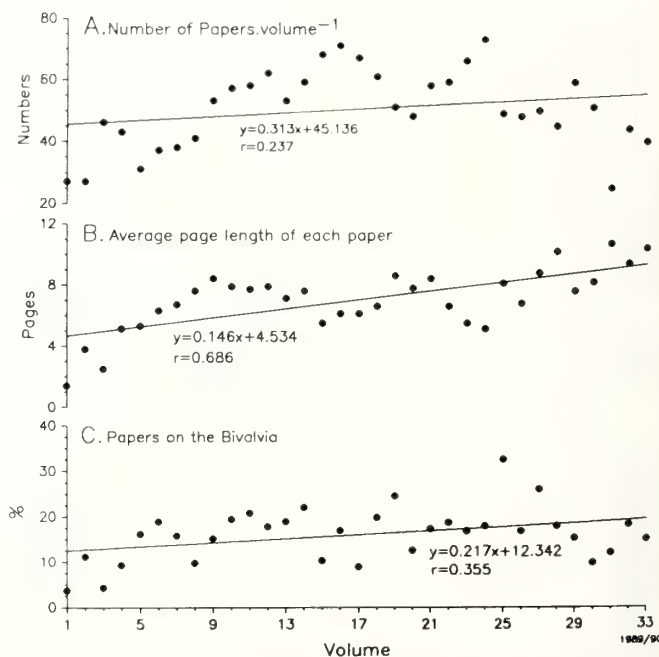


Fig. 2. A, Number of papers; B, average page length of each paper; C, % numbers of papers on the Bivalvia published in *Veliger* (1967-1990).

Table 1. Numbers of papers published on the Mollusca and the three largest classes (plus percentages) between 1969 and 1990 (BIOSIS data).

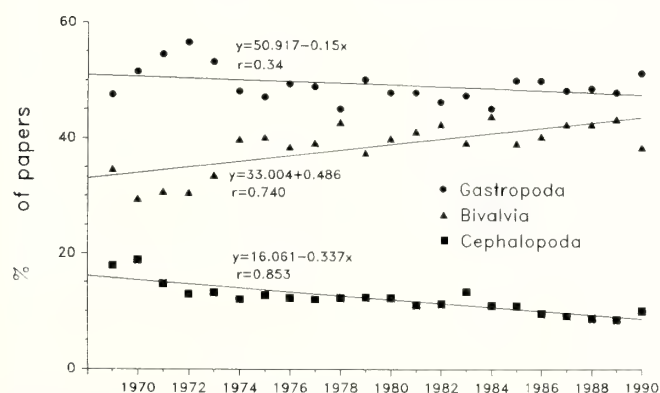
	Mollusca	Gastropoda No.	%	Cephalopoda No.	%	Bivalvia No.	%
1969	379	180	47.5	68	17.9	131	34.6
1970	359	185	51.5	68	18.9	106	29.5
1971	459	250	54.5	68	14.8	141	30.7
1972	524	296	56.6	68	13.0	160	30.5
1973	511	272	53.2	68	13.3	171	33.5
1974	561	270	48.1	68	12.1	223	39.8
1975	669	315	47.1	86	12.8	268	40.1
1976	716	353	49.3	88	12.3	275	38.4
1977	728	355	48.8	88	12.1	285	39.1
1978	717	323	45.0	88	12.3	306	42.7
1979	708	355	50.1	88	12.4	265	37.4
1980	747	357	47.8	92	12.3	298	39.9
1981	829	396	47.8	92	11.1	341	41.1
1982	811	375	46.2	92	11.3	344	42.4
1983	686	325	47.4	92	13.4	269	39.2
1984	833	376	45.1	92	11.0	365	43.8
1985	856	428	50.0	93	10.9	335	39.1
1986	843	422	50.0	81	9.6	340	40.3
1987	880	425	48.3	81	9.2	374	42.5
1988	930	453	48.7	82	8.8	395	42.5
1989	944	453	48.0	81	8.6	410	43.4
1990	801	412	51.4	81	10.1	308	38.5

Since 1974 the percentage contribution of papers on the Gastropoda to the body of molluscan literature has remained relatively stable between 45% and 50%. Since the early 1970's, however, publications on the Bivalvia have increased from around 30% to achieve virtual parity with the Gastropoda at between 40–45%. Thus, the global relative significance of the Bivalvia to working scientists has increased. Relatively more papers are being published on the Bivalvia today, than upon any other molluscan class. Again, I do not wish to make too much of these figures, after all BIOSIS is not comprehensive, but it may be making you think about the remarks made earlier concerning the British and American malacological journals. Do such figures match up

with those obtained from the *Journal of Molluscan Studies* and *The Veliger*? The short answer is that they do not and we thus see that our 'academic' malacological journals are not reflecting, in their contents, the full extent of the research currently being undertaken on the Bivalvia.

I now wish to change the direction and introduce this Symposium but, also, to highlight some areas where I believe more research upon the Bivalvia is needed. I will, however, return to the above figures and arrive at a general conclusion.

The Bivalvia are an ancient group having their origins in the Palaeozoic. *Fordilla* and *Pojetia* are considered to be the oldest bivalves but yet, as Pojeta and Runnegar (1976) point out, we still have not reconstructed an adequate working picture of how such an animal was organized in its shell (Fig. 4). Since the Palaeozoic, the class has radiated, leaving a rich series of fossils and thus a good fossil history that enabled Newell (1965) to lay the basis for a sound, workable, system of classification that, with subsequent minor amendments, serves as a solid base for our understanding of the group's adaptive radiation. A symposium convened in 1977, organized by C. M. Yonge and T. E. Thompson (1978), under the auspices of The Royal Society, attempted, successfully, to marry the works of bivalve palaeontologists and Recent anatomists and we see arising from this venture a much greater appreciation of the need for the two groups of scientists to work together. In this symposium we explore such subjects further in the Evolution and Systematics Session but

**Fig. 3.** Relative numbers of papers published annually on the Gastropoda, Bivalvia and Cephalopoda (BIOSIS data: 1969–1990).

there is, nevertheless, still much scope for closer co-operation between the two groups of scientists.

The researches of Stanley (1986a, b) and Vermeij (1989a, b) among others, have examined the rich *repertoire* of bivalve fossils to present ideas on the causes of marine extinctions and thereby explaining the past and present pat-

terns of geographical restriction. We discuss bivalve biogeography in this Symposium in the Biogeography/Evolution Session. Anatomical studies on the Bivalvia, particularly Recent representatives of what are considered to be ancient lineages, are, however, still needed to help us understand more fully how the Bivalvia has managed to achieve the overall success it so richly, and clearly, enjoys. More importantly, however, such studies would help us to understand the anatomy of the rich array of fossil lineages without Recent representatives. Recently, Purchon (1987) suggested how grades of organization evolved within the Bivalvia to link lineage with lineage, so allowing us to better visualize its evolution. We know much about the shell and ligament structure, mantle fusions and gill, palp and stomach structure of many bivalve families. With continuing expansion and refinement, such information should allow us to make much more intelligent guesses at fossil body structure and cladistics could be a useful tool in this respect.

The adaptive radiation of the Bivalvia, boosted by a new era of success in the Mesozoic, continues to the present day (Stanley, 1977) and Vermeij (1977) and Taylor (1981) have suggested that such a diversification can be correlated with a 'Mesozoic Revolution' involving the adaptive radiation of a new suite of ecologically important predators that exploited the Bivalvia as a major source of food (Fig. 5). Such predation pressure was, thus, deeply felt by the bivalves, effectively driving them underground (Stanley, 1977) with concomitant adaptations for deep burrowing. It could also have fostered the exploitation of rocky shores by more modern heteromyarian heterodonts and the evolution of a coral host/bivalve borer symbiosis and modifications to the borer's shell in tropical species (Fig. 6) (Morton, 1990b). The independent appearance of cementation in many clades of bivalves

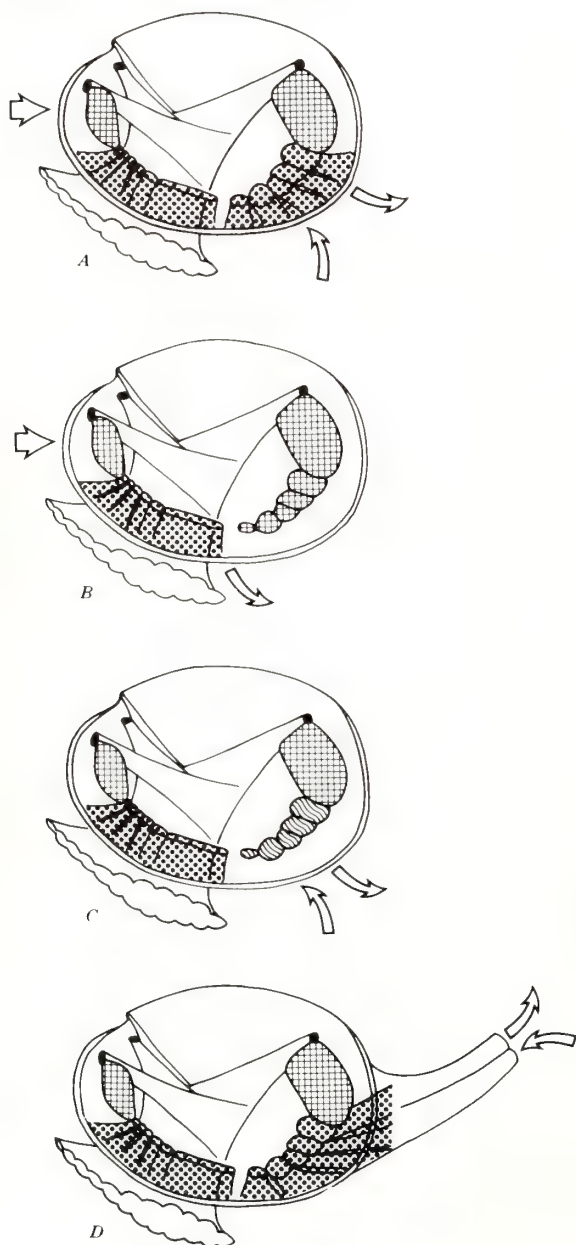


Fig. 4. Four possible explanations of the shell muscle insertions of the Early Cambrian pelecypod *Fordilla*. Adductor muscles are cross hatched; radial pallial muscles are stippled; muscles extending from the shell to the inner surface of the mantle are diagonally shaded; pedal muscle insertions are black. Arrows indicate possible water flow in and out of the mantle cavity. Note that if B were correct, the whole of the posterior end of the shell would be effectively sealed (after Pojeta and Runnegar, 1976).

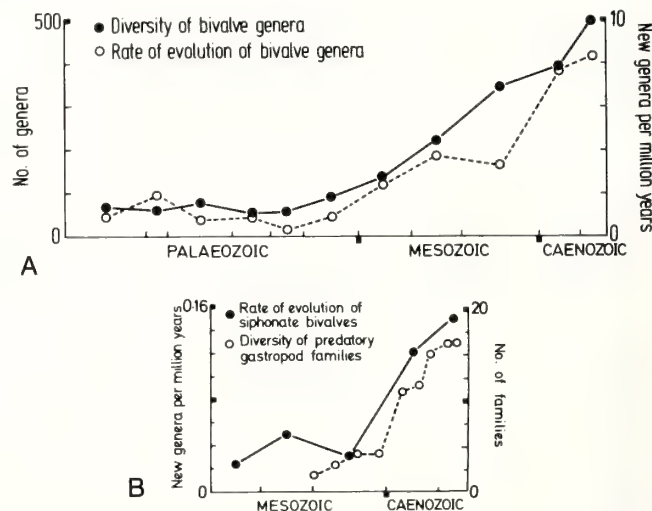


Fig. 5. **A**, rates of bivalve evolution at the family and generic levels (after Stanley, 1973); **B**, rates of bivalve and predatory neogastropod evolution at the family level during the Cenozoic (after Taylor, 1981).

in the Palaeozoic and Mesozoic can also be linked to predation pressure (Harper, 1991). Patterns of evolution in the Bivalvia are beginning to emerge.

Additional evidence, however, points to the success of the Bivalvia and to their importance in aquatic ecosystems. Sanchez-Salazar *et al.* (1987a, b) have shown how on a small sandy beach in North Wales, Great Britain, the cockle *Cerastoderma edule* Linné, is a far more important prey item for foraging crabs [*Carcinus maenas* (Linné)] and Oystercatchers (*Haematopus ostralegus* Linné) than was conceived of previously. On this one small hectare bay, some 18 million juvenile cockles are consumed annually by these predators. Griffiths (1990) has suggested that the same pressures could be acting upon the gregarious bivalves of rocky shores and we can identify a wide range of mesogastropod, neogastropod and opisthobranch snails (*Natica*; *Melongena*, *Buccinum*, *Philine*), crabs (*Carcinus*; *Thalamita*), fish (*Pleuronectes*) and birds (*Haematopus*; *Crocethia*) that exploit bivalves as food. I believe bivalves to be much more important as primary consumers and prey in aquatic food chains than presently appreciated. This could already be recognized, but literature on the subject is published in non-malacological journals (just as with the above papers) such that they become mere names and numbers in papers dealing not so much with the animals themselves but with their presence or absence in communities. In studies of benthic assemblages, they have thus become relegated to a statistic.

I have pointed out (Morton, 1991b) that the Mesozoic Revolution also affected the Bivalvia in other ways, driving some of them into the deep seas where many still retain primitive characters and could thus constitute living fossils. I suggested, for example, that *Batharca* could be a living cryptodont (Morton, 1982) while Allen and Sanders (1969) have suggested that *Nucinella* is a living actinodont. Waller (1971) has suggested that the Propeamussidae are similarly primitive. The deep seas have, however, also fostered the evolution of remarkable structures that have enabled some lineages to pursue predatory careers. I refer of course to the septibranch Anomalodesmata studied by Yonge (1928), Allen (1983), Knudsen (1979), and Reid and Reid (1974) and leading me to suggest (Morton, 1991b) that, in this environment, **they** have become the agents for natural selection rather than a consequence of it. But, how much, in reality do we know of the lifestyle of such animals? If I were to suggest that a group of modern ungulates possessed representatives that are sedentary, ambushing, carnivores residing upon the Himalayas, think of the thousands of researchers who would desperately seek to find them. The mythical Yeti is a case in point. Why not for the septibranchs too then? For that is what they are, in essence, and urgently call out for greater study as very few of them have been studied alive.

There is, I thus believe, still much opportunity for significant research upon the evolution and adaptive radia-

tion of the Bivalvia.

Bivalves are also important economically. The gregarious shallow water representatives of the Pterioda and Heterodonta are two major lineages of bivalves that not only predators exploit as a major class of prey, but which also command our attention as food. Such animals as clams, cockles, mussels, oysters and scallops have been exploited as a human food resource since the Neolithic. Their importance is recognized in their contribution to the composition of the kitchen middens of such early people. Their importance continues today and many are now the subjects of thriving mariculture industries as reflected in the Food and Agriculture Organization (1989) figures for 1987 as presented in Table 2. Such data are, however, in reality, only the tip of a vast underwater iceberg of what the Bivalvia really constitute as a human food resource and their exploitation has led to a massive literature, often unrefereed to by malacologists.

In today's 'economic' world I believe that academic malacologists must surely aim for an enhanced co-operation with mariculturists. We are aware of the researches and mariculture efforts of workers upon the oysters that have, through *Crassostrea gigas* (Ventilla, 1984), revitalized dying industries based upon less hardy species and of the overwhelming success of the Japanese scallop (Ventilla, 1982) and pearl oyster industries, but is there any significant research being undertaken on the wider range of other potentially cultivatable bivalves? One example of such a success has been the effort to re-establish overexploited and thus endangered

Table 2. Fishery statistics for the Mollusca - 1987. (After: Food and Agriculture Organization of the United Nations, 1989).

Commodity	Production (metric tonnes)
Abalone meat (frozen)	1,634
Snails (frozen)	3,964
Oyster meat (frozen)	2,974
Mussel meat (frozen)	11,242
Scallop meat (frozen)	38,866
Clams, cockles etc., meat (frozen)	66,634
Cuttlefish (frozen)	37,831
Squids (frozen)	563,720
Octopus (frozen)	53,183
General cephalopods (frozen)	158,092
General molluscs (frozen)	127,194
Oysters (dried)	3,934
Cuttlefish (dried)	242
Squids (dried)	48,431
Octopus (dried)	80
Squids (smoked)	5,684
General cephalopods (dried, salted, etc.)	15,431
General molluscs (dried, salted, etc.)	8,640
Total	1,147,776
Bivalvia (Total)	123,650

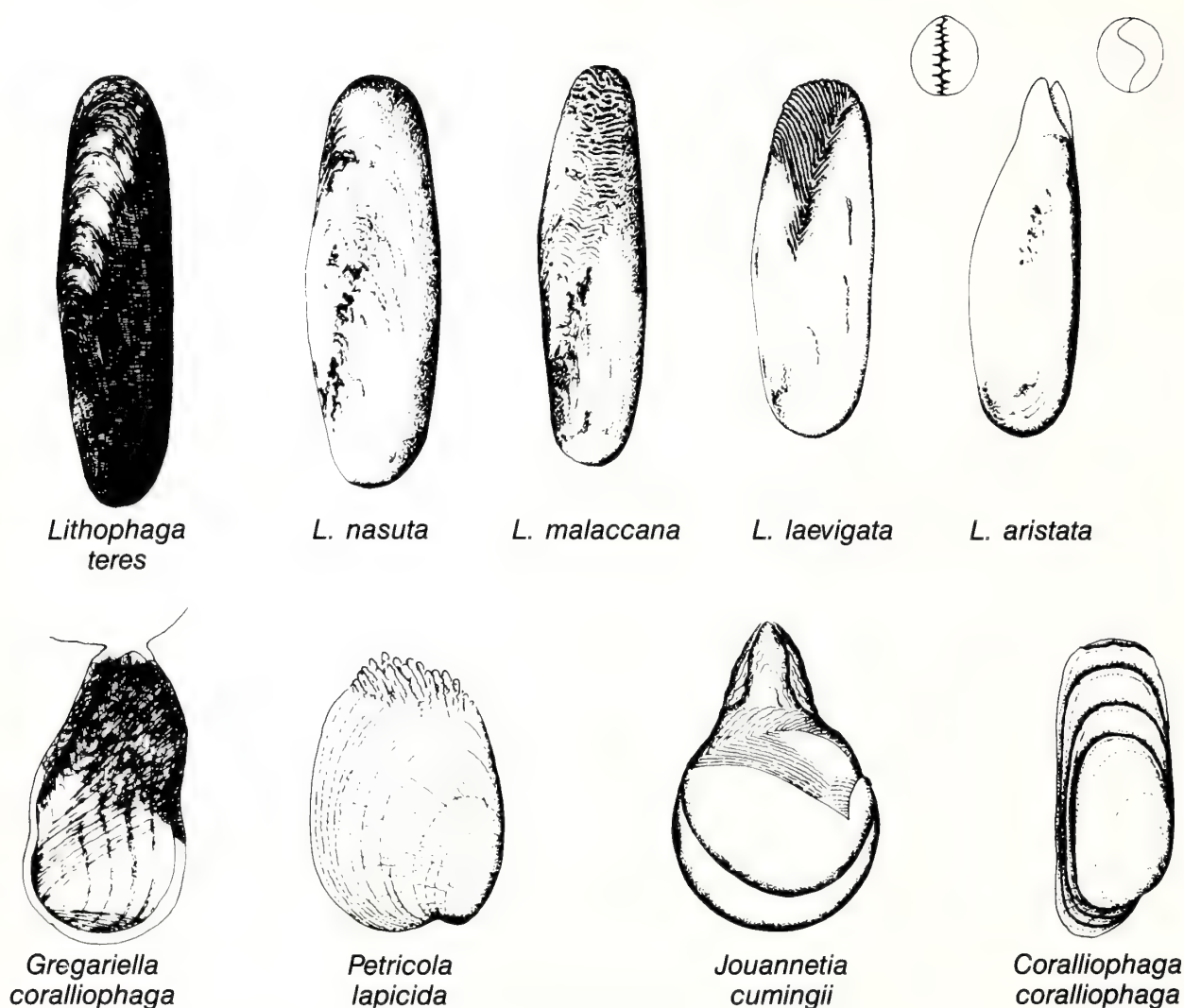


Fig. 6. Lateral views of the shells of coral boring and nestling bivalves. Also illustrated are end-on views of the posterior valve margins of *L. laevigata* and *L. aristata* (after Morton, 1991a).

Pacific island stocks of giant clams. Induced breeding has led not only to the probability of their conservations but also to the commercial development of them as a fishery resource, all the more significant because they do not have to be fed! Bivalves are economically important in other ways: as borers of wood, stone and plastic in the sea, so eloquently evaluated by R. D. Turner and her Harvard school. But, apart from this group, how many others are researching this economically important and fascinating group of bivalves?

We also are aware of the importance of mussels (and oysters) in the monitoring of an almost globally declining in-shore aquatic environment. Their propensity to acquire large amounts of sewage bacteria, red tide toxins and a suite of trace metals and organochlorines, may have reduced the importance of the Bivalvia in the public's eye as potential food (in

the absence of widescale depuration technology) but they are, nevertheless, virtually ideal indicators of environmental stress (Akberali and Trueman, 1985). In polluted Hong Kong, we have identified *Perna viridis* (Linné) (Mytilidae) (Lee, 1985), and, most recently, *Tapes philippinarum* (A. Adams and Reeve) (Veneridae) as highly resistant final indicators of sewage pollution. As such, they dominate polluted hard and soft shores, respectively, adding new significance to the ecological importance of the Bivalvia in such habitats but also posing the question: why is it that we have not exploited such a protein-producing potential for our own benefit? Such animals are also as important, in today's polluted world, as good monitoring sentinels of ambient pollution loadings - but how many such 'mussel watch' networks have been developed, despite their repeated advocacy (National Academy

of Sciences, 1980). I have also to ask why it is that such research, when undertaken, is published not in malacological journals but in their more applied competitors, for example, *Aquaculture* and *Marine Pollution Bulletin*. Academic malacologists seem to have a mental block about the significance of research on the applied aspects of their subject and bivalve workers seem particularly aloof to such work.

Some 'opportunistic' bivalves too are important fouling agents, particularly in fresh waters. I earlier mentioned the subject of my first researches, *Dreissena polymorpha*, spread from its home base in the Caspian Sea throughout Europe during the 19th century Industrial Revolution and on into Great Britain. In 1989 it was introduced into the Great Lakes and, if there are any lessons to be learnt from history, it will invade progressively most of the Americas. The economic consequences of its spread will be enormous and yet I have to ask the question: how many reputable bivalve malacologists have stepped in to research it? My impression is few and, if true, then the story of *Dreissena*, as with *Corbicula flumina* (Müller) and its introduction into North America and subsequent spread (McMahon, 1983), will be left in the hands of engineers and aquatic ecologists whose relationship with the academic malacological fraternity who should be at the forefront of such research, is, at best, tenuous. Regretably, *Dreissena* and *Corbicula* were not discussed at this Symposium, nor even this Conference. Dare I suggest it, but modern bivalve researchers could do no better than to study *Dreissena* and *Corbicula* for they are what we need: living proof of the importance of the Bivalvia and which no gastropod, save vectors of schistosomiasis, can even remotely approach in terms of economic significance. There are many other examples of exotic introductions: *Mytilopsis sallei* Récluz introduced into the Pacific from the Atlantic (Morton, 1987a) and *Musculista senhousia* (Benson) introduced into the southern from the northern Pacific (Willan, 1987). With Pacific trade now outstripping that across the Atlantic, there will be many more such voyages by the Bivalvia (Carlton, 1987). It seems to me that bivalve workers must dirty their hands with such animals that appear, superficially, to be so uninspiring, because, in reality, it is these that are so clearly the visible proof of the success of the Bivalvia in our modern world.

My close friend and colleague, J. C. Britton, and I have, over the years, researched *Corbicula fluminea* here in America and elsewhere. Though others continue to argue with us (and I have no problem with that: arguments lost, won and then lost again are the stuff of science), we believe that only one species has been introduced but that it is polymorphic in terms of shell form, texture and colour and, most fascinating of all, sexual expression (Britton and Morton, 1986). We believe we are essentially seeing, through genotypic and phenotypic plasticity, evolution in action and yet how many other bivalve workers are actively engaged in sorting

out this riddle? Open up any volume of the *Biological Journal of the Linnean Society of London* to see the extent of the literature on polymorphism in the Gastropoda. My own paper on polymorphism in Hong Kong *Corbicula* (Morton, 1987b) was dismissed by a reviewer as wrong - there are simply two species involved - revealing the extent of one of our peers knowledge on this subject in the Bivalvia. I should add that a subsequent paper by Tsoi *et al.* (1990) has demonstrated that there is no significant allozyme difference between the two morphs while Kijviriya *et al.* (1991) have shown, using the same electrophoretic approach, that 21 nominal species of *Corbicula* in Thailand are all assignable to *C. fluminea*. Cain (1988) has recently published a paper on polymorphism in deep-burrowing *Macoma balthica* (Linneé) and suggests that it results from apostatic selection. If this is true, then research on this topic has just received another intellectual injection that should be married into our current concept of how the Bivalvia has evolved and radiated as a result, at least in part, by natural selection through predation.

I am aware of the inherent difficulties in undertaking discrete genetic studies upon randomly externally cross-fertilizing bivalves when the sex of the gamete donors cannot often be determined until autopsy. Such research should, however, be a challenge for us, not a hinderance, and that such animals, gregarious, numerous, prolific, and economically important as well as sessile bioindicators of changing environmental regimes should be receiving far more of our attention than they do currently. With the modern technology of DNA fingerprinting before us, research upon the Bivalvia should, already, have seen a quantum leap in output, but it clearly has not. Even DNA technology is unnecessary, however, for some bivalves, which possess self-fertilizing representatives, e.g. the Galeommatoidea, and O Foighil (1989) has been able to come to important conclusions regarding the significance of planktotrophy versus direct development in the dispersal of representatives of one cosmopolitan genus of this superfamily, *Lasaea*. But there are many other lineages of hermaphroditic bivalves about which virtually nothing is known, for example, the Anomalodesmata (Morton, 1985).

I recently published a paper (Morton, 1991a) in which I argued that life history tactics and reproductive strategies among a suite of bivalves occupying a freshwater to marine continuum were related to the microhabitats occupied (Table 3). This model will, hopefully, be tested by others. I hesitate to ask this, however, but why is it that the Bivalvia so obviously sessile, so obviously in intimate reality with a sweep of aquatic habitats from mountain pools to the abyss have not become *the* model for such studies and further, since we now know that many of their life history traits can be environmentally regulated, that they also become *the* model to help us understand how such natural and unnatural perturbations

Table 3. The sexual strategies adopted by Hong Kong freshwater, estuarine and intertidal bivalves (After Morton, 1991a).

			Sexual strategy adopted				
	Hermaphroditic: (brooding)	Hermaphroditic/ dioecious: Strongly environmentally regulated sex ratio (brooding)	Dioecious: Pronounced female bias (brooding in <i>Anodonta</i>)	Dioecious: Overall female bias; female bias in juveniles (non-brooding)	Dioecious: Slight overall female bias; male bias in juvenile <i>Brachidontes</i> (non-brooding)	Dioecious: (Alternative sexuality in <i>Saccostrea</i>): Slight overall male bias; male bias in juveniles (non-brooding)	Dioecious Slight male bias (not significant) (non-brooding)
Freshwater (small lotic)	<i>Musculium lacustre</i> <i>Pisidium clarkeanum</i> <i>Pisidium annandalei</i>	<i>Corbicula fluminea</i>					
Freshwater (large lentic)			<i>Anodonta woodiana</i> <i>Limnoperna fortunei</i>				
Freshwater (large lotic)				<i>Corbicula</i> cf. <i>fluminalis</i>			
Mangrove					<i>Polymesoda erosa</i>		
Mangrove					<i>Brachidontes variabilis</i>		
Mangrove						<i>Gafrarium pectinatum</i>	
Harbour						<i>Mytilopsis sallei</i> <i>Saccostrea cucullata</i>	
Intertidal marine							<i>Perna viridis</i> <i>Donax semigranulosus</i>

work at the individual, population and species levels.

C. M. Yonge was fascinated by the Bivalvia and laid the foundation for our modern understanding of them. I have read recently the paper by Mikkelsen and Bieler (1989) about the yo-yoing galeommatoidean *Divariscintilla yoyo* Mikkelsen and Bieler. I am also reminded of the work I did upon *Chlamydoconcha orcutti* Dall (Fig. 7A) (Morton, 1981) which is one of the most remarkable of bivalves. It has a minute shell, a weirdly anteriorly monomyarian musculature and very strange defensive appendages. The female also possesses a, possibly parasitic, dwarf male (Fig. 7B). This animal is as exotic as any gastropod. The Galeommatoidea must be one of the most fascinating superfamilies of the Bivalvia. Every study of their numerous representatives speaks of their strange adaptations. Yet, all would agree that the taxonomy and systematics of the group, at every level, are a mess. We desperately need someone to sort them out because I believe that their story is one of the strangest yet to be told. We should

be fascinated by such animals and encourage work upon them for they not only expand our understanding of the full extent of bivalve radiation but also open up new research horizons into the origins, through neoteny, of commensalism and, possibly, parasitism.

Bivalves, alone among the Mollusca, are overwhelmingly economically and ecologically significant and yet they seem, returning to my first figures, to be of declining interest to academic malacologists. Clearly, however, there is not an overall decline in interest (Fig. 3). Is it possible that we as academic malacologists are not in step with globally changing perceptions of the Bivalvia? Is it, further, possible that we are missing a golden harvest of research money and careers that could ultimately be more rewarding? It is clear that few applied aspects of our chosen class were part of this symposium, i.e. their pollution monitoring potential, mariculture significance and their importance as significant fresh water and marine foulers. It could be useful to convene

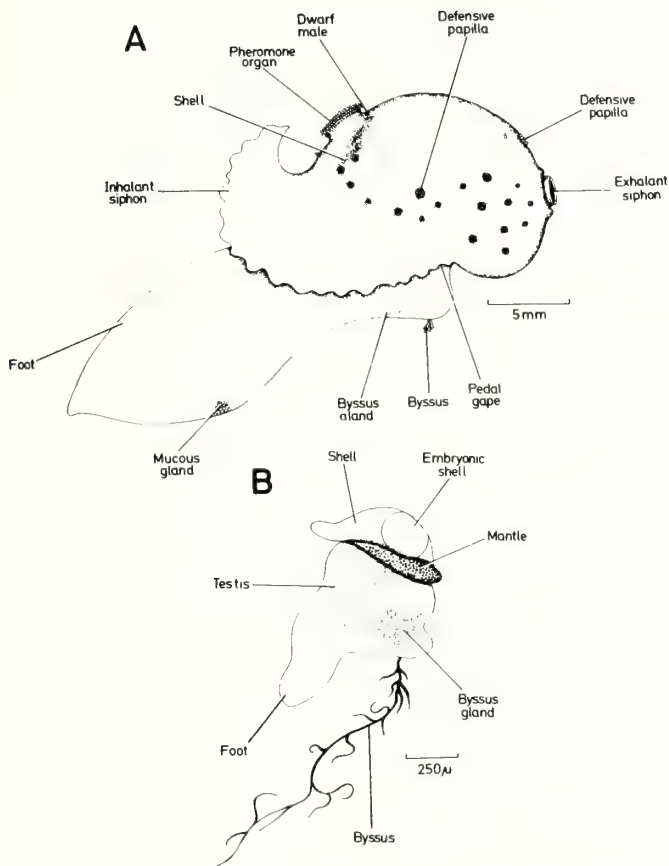


Fig. 7. *Chlamydoconcha orcutti*. A, female; B, dwarf male (after Morton, 1981).

joint meetings of academic and applied bivalve workers on specific topics. I am aware, for example, of the regularly organized pectinid workshops (the 8th was convened in 1991, in Cherbourg, France) that have brought together a rich mix of pure and applied malacologists to great effect. If, therefore, you agree, in any way with my analysis of the current situation with regard to trends and omissions in bivalve research, then perhaps we can persuade the American Malacological Union to assist us in bringing more academic and applied bivalve malacologists together so that the two groups of scientists can begin to benefit, more fully, from the closer co-operation that such meetings would foster.

ACKNOWLEDGMENTS

I am very pleased that Paul Scott and his colleagues seized the initiative in organizing a Symposium and subsequent Workshop on the Bivalvia. I am also grateful to Paul for convening this Symposium and to the Councils of the American Malacological Union and the Western Society of Malacologists for agreeing to host the meeting. I am further grateful that I had the opportunity to address the Opening Session.

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Date of manuscript acceptance: 25 November 1991

The earliest bivalves and their Ordovician descendants

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Abstract. Replicated shell microstructure in a specimen of *Fordilla troyensis* Barrande from the Early Cambrian of Greenland confirms a close relationship between *Fordilla* Barrande from the North Atlantic region and *Pojetaia* Jell from the Early Cambrian of Australia and China. These genera could either be stem-group bivalves which predated the last common ancestor of living members of the class or the earliest known representatives of the extant subclasses Isofilibranchia and Palaeotaxodonta. Two other bivalved molluscs from the Middle Cambrian of Australasia and Scandinavia (*Tuarangia* Mackinnon and *Pseudomyona* Runnegar) had D-shaped valves, a single central adductor muscle, and shells formed of foliated calcite. They have been interpreted as early pteriomorphian bivalves and also as bivalved monoplacophorans; each displays some features of larval bivalves but at shell sizes that are larger than is typical for bivalve prodissoconchs. If *Tuarangia* and *Pseudomyona* were early pteriomorphian bivalves the Class Bivalvia might well be diphyletic. However, it is more likely that the Palaeotaxodonta had a fordillid ancestor and that all other extant subclasses of Bivalvia were derived from this paraphyletic group. Other proposed genera of Cambrian Bivalvia (*Buluniella* Jermak, *Cycloconchoides* Zhang, *Hubeinella* Zhang, *Lamellodonta* Vogel, *Oryzoconcha* He and Pei, *Praelamellodonta* Zhang, *Xianfengoconcha* Zhang, *Yangtzedonta* Yü) are either not molluscs or are junior synonyms of *Fordilla* and *Pojetaia*.

The search for Cambrian ancestors of the bivalves that are so obvious in Ordovician epicontinental marine strata has yielded a variety of fossils that have been offered as early representatives of the Class Bivalvia (Table 1). Of these, only *Fordilla* Barrande (Pojeta, 1975) and *Pojetaia* Jell (Runnegar and Bentley, 1983) are regarded widely as true bivalves but even they could represent stem group taxa in that they could predate the latest common ancestor of all living members of the class (Morris, 1990; Waller, 1990). Two Middle Cambrian taxa, *Pseudomyona* Runnegar and *Tuarangia* Mackinnon (which are obviously related to one another), have been regarded as early pteriomorph bivalves by some workers (Mackinnon, 1982; Berg-Madsen, 1987) but Runnegar (1983) considered them to be bivalved monoplacophorans, analogous to living bivalved opisthobranch gastropods. The purpose of this brief review is to reassess the status of these four genera and their role in the early history of the class. All other proposed Cambrian Bivalvia are either junior synonyms of *Fordilla* and *Pojetaia* or are other kinds of fossils that have been mis-identified as bivalves (Table 1). The most notable of the latter kind is *Lamellodonta simplex* Vogel, which Havlíček and Kříž (1978) showed to be a deformed obolellid brachiopod.

BIOSTRATIGRAPHY OF CAMBRIAN BIVALVES

Both *Fordilla* and *Pojetaia* are middle Early Cambrian

in age. In South Australia, *Pojetaia runnegari* Jell occurs in three of four successive trilobite zones of the middle part of the Early Cambrian (zones of *Abadiella huoi* (Zhang), *Pararaia tatei* (Woodward) and *P. janeae* Jell; Bengtson *et al.*, 1990). This stratigraphic range is approximately equivalent to the Chiungchussu and Tsanglangpu stages of the Chinese Early Cambrian succession and to the Atdabanian and Botomian stages of the Siberian Platform. Chinese occurrences of *Pojetaia* are from the early Tsanglangpu stage (Chen and Wang, 1985; He and Pei, 1985) and are probably equivalent in age to the zone of *Nevadella* Raw of North America (P. A. Jell, pers. comm.).

Fordilla is found in the middle Early Cambrian rocks in eastern North America, Greenland and Denmark (Pojeta, 1975) and in Tommotian and Atdabanian strata of the Siberian Platform (Jermak, 1986, 1988). There is no longer any evidence that the first appearance of *Pojetaia* predates significantly the origin of *Fordilla* (e.g. Jell, 1980); the two taxa probably originated at approximately the same time and then coexisted in different biogeographic regions (Redlichian and Olenellian realms; Kobayashi, 1972) for about 10 million years.

Tuarangia paparuia Mackinnon and *T. gravgaerdensis* Berg-Madsen occur in approximately coeval late Middle Cambrian (Boomerangian) strata in New Zealand and Denmark (Berg-Madsen, 1987). *Pseudomyona* is known only from its type locality in northwestern Queensland which is early Middle Cambrian (Floran) in age (Southgate, 1986).

Table 1. List of generic and specific names that have been proposed for fossils considered to be Cambrian bivalves (type species are identified by an asterisk). Only two Early Cambrian species (*Fordilla troyensis* and *Pojetaia runnegari*) are certainly bivalves. Our assessment of the remaining taxa, based in most cases upon an examination of original material, is given in the right hand column.

<i>Buluniella</i> Jermak, 1986	
<i>B. borealis</i> * Jermak, 1986	<i>Fordilla troyensis</i> ?
<i>Cycloconchioides</i> Zhang, 1980	
<i>C. elongatus</i> Zhang, 1980	stenothecoid
<i>C. venustus</i> Zhang, 1980	stenothecoid
<i>Fordilla</i> Barrande 1881	
<i>F. troyensis</i> * Barrande, 1881	Early Cambrian bivalve
<i>F. sibirica</i> Krasilova, 1977	<i>F. troyensis</i>
<i>Hebeinella</i> Zhang, 1980	
<i>H. formosa</i> * Zhang, 1980	stenothecoid
<i>Lamellodonta</i> Vogel, 1962	
<i>L. simplex</i> * Vogel, 1962	inarticulate brachiopod
<i>Oryzoconcha</i> He and Pei, 1985	
<i>O. prisca</i> * He and Pei, 1985	<i>Pojetaia runnegari</i>
<i>Pojetaia</i> Jell, 1980	
<i>P. ovata</i> Chen and Wang, 1985	<i>P. runnegari</i>
<i>P. runnegari</i> * Jell, 1980	Early Cambrian bivalve
<i>Praelamellodonta</i> Zhang, 1980	
<i>P. elegans</i> * Zhang, 1980	stenothecoid
<i>Pseudomyona</i> Runnegar, 1983	
<i>Myona queenslandica</i> * Runnegar and Jell, 1976	Middle Cambrian bivalve?
<i>Tuarangia</i> Mackinnon, 1982	
<i>T. paparua</i> * Mackinnon, 1982	Middle Cambrian bivalve?
<i>T. graygaerdensis</i> Berg-Madsen, 1987	Middle Cambrian bivalve?
<i>Xianfengconcha</i> Zhang, 1980	
<i>X. elliptica</i> Zhang, 1980	stenothecoid
<i>X. rotunda</i> Zhang, 1980	stenothecoid
<i>X. minuta</i> Zhang, 1980	stenothecoid
<i>Yangtzedonta</i> Yü, 1985	
<i>Y. primitiva</i> * Yü, 1985	unique, unidentified microfossil

Berg-Madsen (1987) also illustrated a single specimen of *Tuarangia* from a glacial erratic in north Poland; the presence of the conodont *Westergaardodina tricuspidata* Müller in the same boulder suggested an early Late Cambrian age for the source rock.

HIGHER TAXA OF CAMBRIAN BIVALVES

When Jell (1980) described *Pojetaia runnegari* he noted its similarities to *Fordilla troyensis* (size, shape, cardinal hinge teeth, opisthodontic ligament, etc.) and placed it in the same family (Fordillidae) and order (Fordilloida) as *Fordilla*. In contrast, Runnegar and Bentley (1983) emphasised similarities between *Fordilla* and Ordovician isofilibranch bivalves such as *Neofordilla* Krasilova and *Modiolodon* Ulrich and they therefore referred *Fordilla* to the Mytilacea. *Pojetaia*, on the other hand, was regarded as a primitive nuculoid palaeotaxodont. This interpretation placed the origin of these two

subclasses (Isofilibranchia and Palaeotaxodonta) within the Fordillidae during the Early Cambrian.

One of the most distinctive features of *Pojetaia* is seen on phosphatic internal molds of the shell (Figs. 1D-E). The surface of most internal molds is covered with imbricated polygonal cells that are inclined in a consistent way and become smaller toward the growing margin of the valve. These cells were interpreted as the impressions of the ends of near-vertical prisms formed of aragonite fibers by Runnegar and Bentley (1983) and Runnegar (1985), but this interpretation has been challenged by Carter and Clark (1985) and Carter (1990), who regard the structure as the imprints of exceptionally large (30 µm) tablets of nacre. Although we do not agree with this suggestion because nacre tablets are never imbricated, we must admit that the microstructure of the *Pojetaia* shell is not yet well understood.

Nevertheless, a clearly homologous microstructure was present in the *Fordilla* shell (Figs. 1A-B). A steinkern of *F. troyensis* discovered in Greenland by John S. Peel has the same kind of cellular network as internal molds of *Pojetaia*, except that in *Fordilla* the cells are more elongated than they are in *Pojetaia*. As in *Pojetaia*, each cell has closely-spaced transverse marks which could be casts of individual mineral (aragonite?) fibers.

The importance of this unusual shell microstructure is that it reunites *Fordilla* and *Pojetaia* into a monophyletic group (Fig. 2). Thus Waller (1990) treated this microstructure as an autapomorphy of the Fordilloida and, on that basis, regarded the fordillids as an extinct stem group which diverged from the line leading to modern bivalves prior to the origin of the class. In Waller's phylogenetic tree all modern bivalve higher taxa are derived directly or sequentially from Early Ordovician palaeotaxodonts.

It is unlikely that the distinctive shell microstructure of *Fordilla* and *Pojetaia* would be lost independently in lines leading to both the Palaeotaxodonta and the Isofilibranchia so the independent derivation of these subclasses from the Fordillidae is not supported by the new microstructural evidence. However, inclined (but near-vertical) aragonitic prisms of the type postulated by Runnegar and Bentley (1983) in *Pojetaia* have been observed in the outer shell layer of a Devonian nuculoid [*Palaeoneilo filosa* (Conrad); Carter, 1990: 159-162] and so it is still possible that the Early Ordovician palaeotaxodonts are direct descendants of a fordillid such as *Pojetaia*.

A quite different shell microstructure unites *Tuarangia* and *Pseudomyona* (Fig. 3G). Once again, this microstructure is known only from phosphatic replicas of the inner surfaces of recrystallized shells. It has been interpreted as replicated foliated calcite by comparison with modern examples and crystallographic analysis (Mackinnon, 1982; Runnegar, 1984); it must be a primary feature of the shell rather than a secondary diagenetic artefact for the foliated calcite

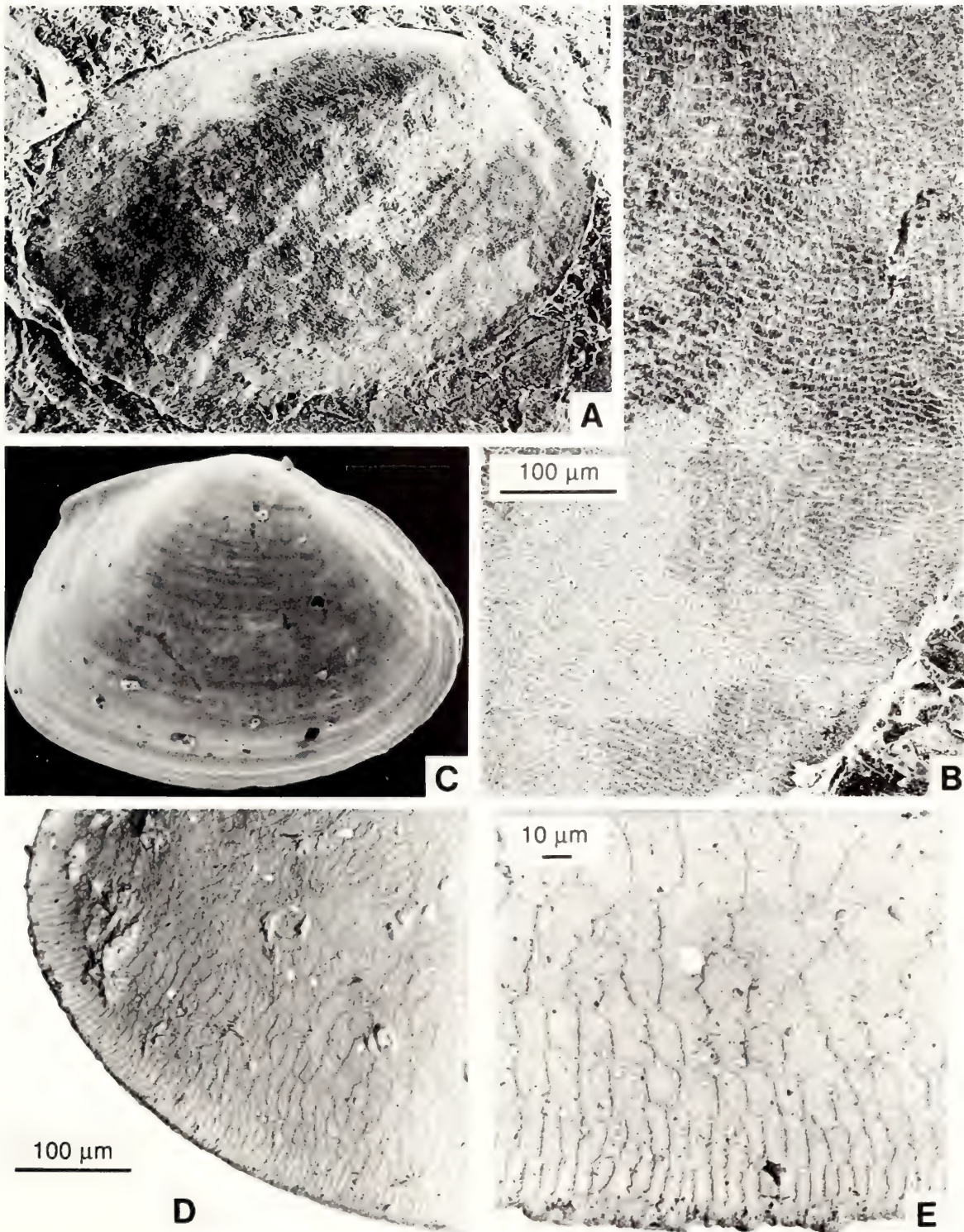


Fig. 1. Replicated shell microstructure of *Fordilla troyensis* Barrande (A-B) and *Pojetaia runnegari* Jell (C-E). Early Cambrian bivalves. **A**, dolomitic internal mold of right valve, Early Cambrian, Greenland, showing replicas of structures interpreted by Runnegar and Bentley (1983) as casts of the ends of composite prisms formed of fibrous aragonite (shell length = 3.25 mm). **B**, enlargement of antero-ventral part of A. **C**, exterior of right valve; shell is 1.0 mm long. **D-E**, scanning electron micrographs of the antero-ventral part of internal mold of right left valve; the images have been electronically inverted to give the impression of the actual structure rather than its negative cast.

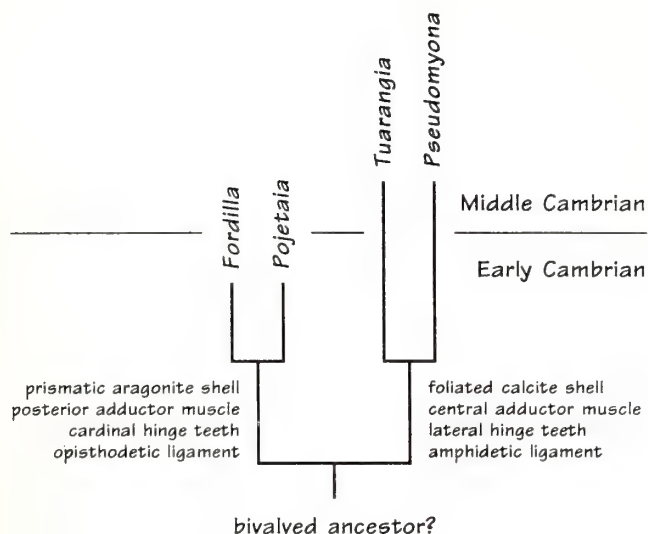


Fig. 2. Tree illustrating the phylogenetic hypotheses discussed in the text. It is concluded that *Fordilla* Barrande and *Pojetaia* Jell did not share a common bivalved ancestor with *Tuarangia* Mackinnon and *Pseudomyona* Runnegar.

stops abruptly at the edges of muscle scars (Fig. 3F) and the trend of the folia is related to their position along the valve margin (Runnegar, 1983). A similar microstructure has been observed in the Middle Cambrian snorkel-bearing univalve *Eotebenna* Runnegar and Jell (Runnegar and Jell, 1976; Runnegar, 1983) but is otherwise known from fossil molluscs until the (presumably independent) origins of foliated calcite in the patelloid limpets and pectinoid bivalves some time during the early post-Cambrian Paleozoic.

In addition to a foliated calcite shell, *Tuarangia* and *Pseudomyona* each had an amphidetic hinge, D-shaped valves and lateral but not cardinal teeth (Fig. 3). They both probably also possessed the central adductor muscle seen in *Pseudomyona* (Runnegar, 1983). However, it is not clear whether the "protoconch" of *Pseudomyona* (Figs. 3A, 3C-D) is homologous with the central "ligament pit" of *Tuarangia* (Fig. 3E).

Mackinnon (1982) and Berg-Madsen (1987) have regarded *Tuarangia* and *Pseudomyona* as early pteriomorphian bivalves belonging to the extinct Order Tuarangiida. They cited the amphidetic ligament, straight hinge, lateral teeth and foliated calcite shell as pteriomorphian characters. The problem with this interpretation is that this collage of characters is not characteristic of the early pteriomorphians so far discovered in Ordovician strata (Pojeta and Runnegar, 1985).

DISCUSSION

By the end of the Ordovician, at least five of the extant subclasses of bivalves had appeared: Palaeotaxodonta;

Isofilibranchia [Boss (1982) and Waller (1990) consider the Isofilibranchia to be a superorder of the Pteriomorphia]; Anomalodesmata; Heteroconchia; Pteriomorphia (Pojeta and Runnegar, 1985). On both stratigraphic and morphological grounds the palaeotaxodonts are regarded frequently as primitive and the direct or indirect ancestors of both the Pteriomorphia and the Heteroconchia (Palaeoheterodonta + Heterodonta) (Pojeta and Runnegar, 1985; Waller, 1990). It is possible (but less likely) that the nuculoid palaeotaxodonts were derived from an actinodont heteroconch (Babin and Gutiérrez-Marco, 1991).

Waller (1990) used the distinctive microstructure of *Pojetaia* and *Fordilla* as a synapomorphy for the Fordilloidea, which he regarded as the sister group of the rest of the Bivalvia. He supported this taxonomic decision with the assumption that the fordillids had lost a preexisting nacreous inner shell layer and had not yet acquired a fibrous layer in their ligament. This allowed him to reinstate the palaeotaxodonts as the earliest members of the crown group. Morris (1990) came to a similar conclusion but for less explicit reasons.

Although some laterally-compressed Cambrian molluscs apparently had prismatic-nacreous aragonitic shells (Runnegar, 1985), there is no evidence that nacre was present in (or absent from) the ribeirioid rostroconch ancestors of the bivalves (Runnegar, 1983). Similarly, the ligaments of *Pojetaia* and *Fordilla* are not preserved and so could have been fibrous, granular or unmineralized; in any case, the ligament of nuculids is not fibrous and Waller (1990) has therefore suggested that the granular ligament of *Nucula* Lamarck is a derived condition. [Based on evidence obtained from well-preserved Devonian nuculoids, Carter (1990) also proposed that the granular ligament of *Nucula* is derived from an ancestral weakly-mineralized to non-mineralized condition.] Given these and other uncertainties we tentatively maintain the fordillids within the crown group for the time being. As mentioned above, it is possible that *Pojetaia* was an early palaeotaxodont.

Pojeta and Runnegar (1985) recognized four major kinds of Ordovician pteriomorphs: pterineid pteriaceans; cyrtodontids; ambonychiids; a probable ancestral limid, *Prolobella*? Ulrich. Under some existing classifications the pterineids and the limid would be placed together in the order Pterioidea but this grouping makes little sense in an Ordovician context because the pectiniform shell of *Prolobella*? is unlikely to be homologous with the shells of younger Pectinacea. We therefore agree with Waller (1978) and Johnston (1991) who assigned the ambonychiids and limoids to the superorder Prionodonta, *sensu* Boss (1982).

The duplivincular ligament of the pterineids, cyrtodontids, and ambonychiids strongly suggests that they constitute a monophyletic group (Prionodonta + Eupteriomorphia). Many authors have considered the cyrtodontids to

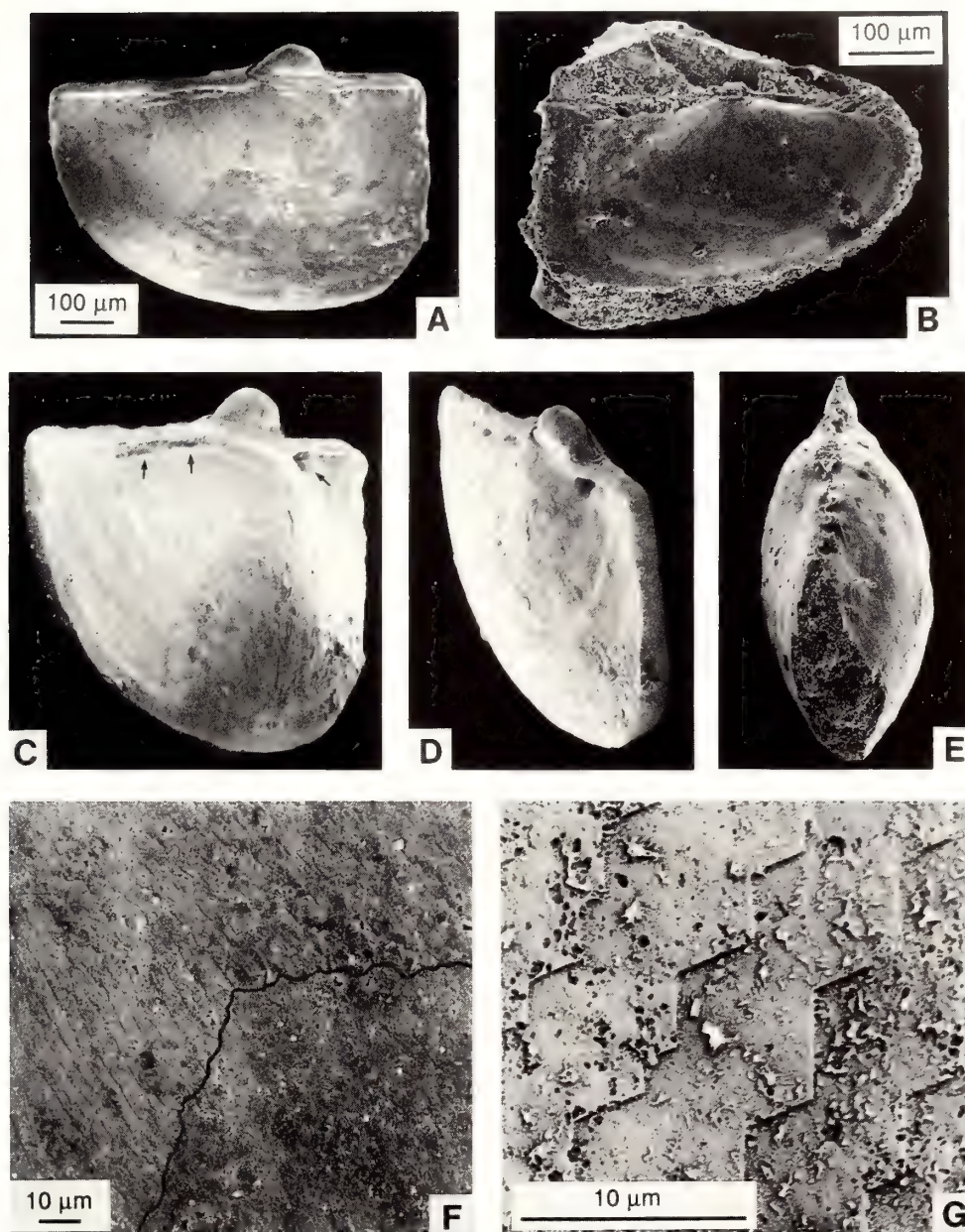


Fig. 3. Middle Cambrian bivalved molluscs, *Pseudomyona queenslandica* (Runnegar and Jell) (A, C-D, F-G) and *Tuarangia gravgaerdensis* Berg-Madsen (B, E). **A, C-D**, phosphatic internal mold viewed from right? side and posterior? and anterior? ends; note lateral teeth (arrows) and univalved protoconch. **B**, internal mold of right? valve showing well-developed lateral teeth. **E**, end-on view of internal mold showing crenulations caused by interlocking teeth and central "ligament pit". **F**, surface of internal mold showing casts of overlapping calcite folia (upper left), edge of adductor muscle scar (ink line), and smooth surface of adductor scar; polygons near edge of adductor scar are interpreted as casts of myostracal prisms. **G**, enlargement of replicated foliated calcite on surface of internal mold.

be the most primitive pteriomorphs and they derive them either from the cycloconch actinodonts (Pojeta and Runnegar, 1985; Johnston, 1991) or "actinodont" palaeotaxodonts (Waller, 1990). Johnston (1991) has described a Silurian eupteriomorph (*Umburra cinefacta* Johnston) which he regards as "more primitive dentally" than any known cyrtodontid. If this were true, then the common ancestor of the

prionodonts and the eupteriomorphs would have had an equivalved shell, a duplivincular ligament and "actinodont" hinge teeth. Although this hypothetical ancestor approximates some of the morphological features of *Tuarangia* and *Pseudomyona*, it almost certainly would have had anterior and posterior adductor muscles (Johnston, 1991) rather than the single central adductor muscle of *Pseudomyona* and a

nacreous or crossed-lamellar aragonitic shell (Carter, 1990) instead of the foliated calcite shell of *Pseudomyona* and *Tuarangia*. Thus the postulated genetic connection between *Tuarangia*/*Pseudomyona* and the Pteriomorpha (Mackinnon, 1982; Berg-Madsen, 1987) remains tenuous and is not supported by the evidence currently available. As there is no character apart from the bivalved condition in common between the fordillids and either *Pseudomyona* or *Tuarangia* it is difficult to sustain the hypothesis that they once shared a common bivalved ancestor (Fig. 2). On the contrary, the presence of foliated calcite in the pseudobivalved univalve *Eotebenna pontifex* Runnegar and Jell (Runnegar and Jell, 1976) is an indication that *Pseudomyona* and *Tuarangia* are not true bivalves and that the Class Bivalvia is therefore monophyletic.

ACKNOWLEDGMENTS

We thank Dr. J. S. Peel, Geological Survey of Greenland, Copenhagen for the specimen of *Fordilla troyensis* illustrated in figure 1 and Dr. Vivianne Berg-Madsen, Geological Institute, Stockholm University for the photographs of *Tuarangia gravgaerensis*. Mr. Paul Belasky kindly translated some of the Russian papers. Dr. Joseph G. Carter and an anonymous reviewer provided helpful suggestions for improvements to the manuscript.

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Date of manuscript acceptance: 16 January 1992

Systematics evolution and distribution of mussels belonging to the genus *Mytilus*: an overview

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Abstract. Despite their scientific and commercial interest and their widespread distribution throughout the cooler waters of both northern and southern hemispheres, the taxonomy of mussels belonging to the genus *Mytilus* remains controversial. This paper reviews the systematics of this group, albeit with particular emphasis on the smooth-shelled mussels of the *M. edulis* complex, and stresses throughout the need for a multidisciplinary approach. Multivariate analysis of allozyme and morphometric data obtained for mussels worldwide now provides compelling evidence for the existence of three distinct evolutionary lineages: *M. edulis*; *M. galloprovincialis*; *M. trossulus*. No single taxonomic character discriminates unequivocally among these taxa though certain characters, either individually or in combination, are virtually diagnostic. All three lineages occur in northern waters but only *M. edulis* and *M. galloprovincialis* have so far been recorded from the southern hemisphere. Whether these taxa are accorded full specific status will require an agreed operational definition of biological species. Future research should focus on the biological mechanisms that maintain the distinctive characteristics of these mussels across vast distances despite the occurrence of hybridisation and the massive potential for larval dispersal. The origin, evolution and distribution of mussels within the genus are discussed.

The genus *Mytilus* is one of the most cosmopolitan of all marine genera, occurring at higher latitudes in all oceans and major seas of both northern and southern hemispheres. It is found intertidally and subtidally, in estuarine and fully saline habitats, attached by means of byssal threads to a wide variety of hard or semiconsolidated substrata. In view of its widespread distribution, as well as its scientific and commercial importance, it is perhaps surprising that the taxonomy and systematics of this extensively studied genus still remains a somewhat controversial issue (e.g. Gosling, 1984; McDonald *et al.*, 1991).

Much of the early taxonomy of *Mytilus* was based solely on morphological features, particularly those pertaining to the shell. However, ontogenetic and environmentally induced variation in shell characteristics (e.g. Seed, 1968, 1973, 1978; Lewis and Seed, 1969), combined with the complex interactions that are now known to exist among several taxa within this genus, has produced an extremely confused and largely erroneous taxonomy (Koehn, 1991). In a comprehensive review of the genus, Lamy (1936) recognised the following smooth-shelled mussels as distinct species: *M. edulis* Linnaeus from north temperate waters; *M. galloprovincialis* Lamarck from the Mediterranean Sea; *M. trossulus* Gould from the Pacific coast of North America, *M. chilensis* Hupe' and *M. platensis* Orbigny from the east and west coasts of South America respectively; *M. planulatus* Lamarck from Australia and New Zealand. He also described *M. desolationis* Lamy (= *M. kerguelensis* Fletcher) from the Kerguelen islands in the southern Indian Ocean. These taxa, however,

were reported by Soot Ryen (1955) as geographical subspecies or races of the *M. edulis* species complex. Other taxa previously considered to be subspecies of *M. edulis* include the Californian bay mussel, *M. diegensis* Coe (Soot Ryen, 1955) and *M. aoteanus* Powell from New Zealand (Fleming, 1959) together with *M. kussakini* and *M. zhirmunskii* from the Pacific coast of Asia (Scarlato and Starobogatov, 1979).

Mytilus californianus Conrad, a distinctively different species of large body size and divergent ecology to *M. edulis*, is identified readily by the presence of radiating ribs on the shell (Soot Ryen, 1955). *M. coruscus* Gould (= *M. crassitesta* Lischke) is a thick-shelled, ribbed mussel with minute crenulations along the ventral margin close to the apex (Kira, 1962). Unfortunately, however, we have little or no detailed information regarding this mussel and its systematic status thus remains uncertain. Recently, Vermeij (1989) has speculated that *M. californianus* and *M. coruscus* could in fact comprise a single species with geographical variations in the prominence of the radiating ribs. However, because both of these mussels are distinguished easily from the smooth-shelled mussels of the *M. edulis* group, they will not be considered in any detail in this paper.

The use of enzyme electrophoresis to characterise individual and population differences in genetic composition, together with multivariate techniques applied to both enzyme and morphometric phenotypes, have assisted greatly in elucidating the systematics and taxonomic status of species of smooth-shelled mussels (e.g. McDonald and Koehn, 1988; Varvio *et al.*, 1988; McDonald *et al.*, 1991). Although three

taxa have been identified (*Mytilus edulis*, *M. galloprovincialis* and *M. trossulus*), hybridisation has been reported at most locations where the ranges of these mussels coincide and consequently this has led to considerable speculation regarding their taxonomic status (e.g. Skibinski *et al.*, 1983; Gosling, 1984, 1992; McDonald and Koehn, 1988; Johannesson *et al.*, 1990; Väinölä and Hvilsom, 1991). In this paper I shall document briefly the evidence for the existence and distribution of these three relatively distinct mussels, albeit with particular emphasis on the taxonomic validity of the Mediterranean mussel *M. galloprovincialis*, which was originally thought to be restricted to European coasts but which now appears to be far more widely distributed (e.g. Wilkins *et al.*, 1983; Lee and Morton, 1985; Grant and Cherry, 1985; McDonald and Koehn, 1988; McDonald *et al.*, 1991). Much less information is available currently concerning the systematics, distribution and ecological characteristics of *M. trossulus*.

SYSTEMATIC CHARACTERISATION OF MYTILUS

A) ENZYME ELECTROPHORESIS: Allozyme characters have assisted greatly in clarifying the complex biosystematics of the genus *Mytilus*. Despite the large number of enzymes that are potentially available for study, in practice only a few have sufficiently high levels of variation to be of significant taxonomic value (e.g. Ahmad *et al.*, 1977). Earlier studies on the *M. galloprovincialis*-*M. edulis* complex (reviewed by Gosling, 1984) used various combinations of six loci; esterase D (*Est-D*), leucine aminopeptidase (*Lap-1*), glucose phosphate isomerase (*Gpi*), aminopeptidase (*Ap*), peptidase 2 (*Lap-2*) and phosphoglucosmutase (*Pgm*). More recently, octopine dehydrogenase (*Odh*) and mannose phosphate isomerase (*Mpi*) have also been incorporated into the suite of enzymes used to differentiate between these smooth-shelled mussels (e.g. Skibinski, 1983; Grant and Cherry, 1985; Varvio *et al.*, 1988; McDonald and Koehn, 1988). None of these loci, however, discriminate unequivocally between *M. edulis* and *M. galloprovincialis*, but according to Varvio *et al.* (1988) the *Mpi* locus is 'virtually diagnostic'. McDonald and Koehn (1988) similarly found that *Mpi* was diagnostic in almost all the allopatric populations of *Mytilus* that they studied although a combination of other loci with large differences in allele frequency could also effectively discriminate between different taxa. When a combination of four allozyme loci were used, Sanjuan *et al.* (1990) found that the probability of misclassification was exceedingly low (1.5×10^{-7}); indeed 99% of all individual mussels in their samples could be assigned correctly on the *Est-D* genotype alone. Although a less well studied enzyme, leucyl glycyl glycine peptidase is also reported to provide an almost perfect discrimination between *M. edulis* and *M. galloprovincialis* (Grant and Cherry, 1985). The principal loci used in studies of *Mytilus* genetics, especially those which have proved to be most

valuable in taxonomic studies, are comprehensively reviewed by Gosling (1992).

Beaumont *et al.* (1989) examined allele frequencies at three loci (*Est-D*, *Mpi*, *Odh*) in mixed populations of *Mytilus edulis* and *M. galloprovincialis* from two physically contrasted sites, Rock and Polzeath, in the Camel estuary in south-west England, and a pure population of *M. galloprovincialis* from Langebaan lagoon on the west Cape coast of South Africa. Their results, summarised in Table 1, reveal markedly different allele frequencies between these two mussels, particularly with respect to the *Est-D* and *Mpi* loci. At Rock, the *Mpi* locus proved to be less effective at differentiating *M. edulis* and *M. galloprovincialis* than the *Est-D* locus, whilst both of these loci were rather poor discriminators in the South African and Polzeath populations. *Odh* genotypes did not appear to be particularly good discriminating characters in any of the populations studied though the data of Varvio *et al.* (1988) did allow clear discrimination of *M. galloprovincialis* populations on the basis of *Odh* allelic composition. A further feature of the Rock mussel population was the disparity in the percentage of *M. edulis* compared to *M. galloprovincialis* that were misidentified by the *Mpi* locus. This is owing to the fact that the *Mpi*⁶³ allele (the characteristic *M. galloprovincialis* allele) was present in the *M. edulis* population at a frequency of 0.197 but the reverse was not true as the *M. edulis* allele, *Mpi*¹⁰⁰, was present in the *M. galloprovincialis* population at a frequency of only 0.053 (Table 1).

Two salient features would thus appear to emerge from the use of single locus genotypes as characters for discriminating between *Mytilus edulis* and *M. galloprovincialis*. Firstly, a locus can give good discrimination in one

Table 1. Allele frequencies at three loci in sympatric populations of *Mytilus edulis* and *M. galloprovincialis* from Rock and *M. galloprovincialis* from Polzeath and South Africa (after Beaumont *et al.*, 1989).

Locus	Alleles (relative mobility)	Allele frequencies			
		Rock <i>M. edulis</i>	<i>M. gall.</i>	S. Africa <i>M. gall.</i>	Polzeath <i>M. gall.</i>
<i>Esterase-D</i> (<i>Est-D</i>)	60	0.014	—	—	—
	82	0.021	0.941	0.802	0.360
	100	0.936	0.059	0.198	0.640
	118	0.029	—	—	—
<i>Mannose</i> <i>phosphate</i> <i>isomerase</i> (<i>Mpi</i>)	63	0.197	0.947	0.882	0.722
	100	0.796	0.053	0.118	0.278
	133	0.007	—	—	—
<i>Octopine</i> <i>dehydro-</i> <i>genase</i> (<i>Odh</i>)	60	0.007	0.006	—	0.015
	70	0.111	0.530	0.540	0.634
	77	0.014	—	0.030	—
	100	0.799	0.226	0.120	0.227
	106	0.014	0.006	—	0.015
	112	0.055	0.232	0.310	0.109

population but poorer discrimination in another, and, secondly, a locus may be diagnostic for one species, but not the other, within any single mussel population (Beaumont *et al.*, 1989). Variations in genotype, whether on a local or geographical scale, could of course reflect differential patterns of environmental selection rather than distinct evolutionary backgrounds (e.g. Murdock *et al.*, 1975; Koehn *et al.*, 1980; Gartner-Kepkay *et al.*, 1983; Johannesson *et al.*, 1990; Tedengren *et al.*, 1990). The marked differences in allele frequencies reported for the mussel populations at Rock, however, cannot easily be attributed to such causes since these mussels occur within mixed clumps and are thus presumably subjected to identical environmental conditions.

On the basis of five allozyme loci, Koehn *et al.* (1984) were able to separate samples of putative *Mytilus edulis* from several sites throughout eastern North America into three distinguishable groups, though one of these involved separation at a single locus (*Lap*) and was not, therefore, thought to represent a distinct taxonomic group. The other two groups, however, were very different at several loci and this led these authors to suggest that one of these groups represented a hitherto unrecognised species. Subsequently, this was given additional support by Varvio *et al.* (1988) who showed that this mussel was most similar to *Mytilus* from the Baltic Sea (see also Bulheim and Gosling, 1988) and which is now recognised as *M. trossulus*, a species reported previously only from parts of the Pacific coast of North America (e.g. McDonald and Koehn, 1988).

More recently McDonald *et al.* (1991) used a multivariate technique to analyse the electrophoretic data at eight loci in over a thousand mussels collected from a total of 45 sites in the northern and southern hemispheres. Allozyme data (71 characters) were reduced and displayed using principal component analysis which locates the orthogonal axes accounting for the greatest amount of variation in the multidimensional space. This analysis defined three distinct clusters of individuals in the northern hemisphere samples but only two clusters in southern hemisphere mussels, albeit with some intermediate individuals mainly from those sites where these mussels come into contact and hybridisation occurs (Figs. 1A,B). Each cluster in the northern hemisphere could be assigned to an extant species, *Mytilus edulis*, *M. galloprovincialis* or *M. trossulus*, based on the examination of principal component scores of individuals from locations where the identity of mussels had been designated previously (e.g. McDonald and Koehn, 1988; McDonald *et al.*, 1990).

The *Mytilus edulis* cluster in the southern hemisphere, comprising mussels from South America, the Falklands and Kerguelen islands (= "South American mussels"), were most similar to northern hemisphere *M. edulis*, although they did contain alleles that were characteristic of all three northern mussels. The reason for this is that many loci of these

"South American" mussels contained alleles that in northern hemisphere mussels were common only in *M. galloprovincialis* or *M. trossulus*. Blot *et al.* (1988) has similarly shown that mussels from the Kerguelen islands were more similar genetically to northern *M. edulis* than to *M. galloprovincialis*. Mussels from Australia, Tasmania and New Zealand (= "Australian mussels") formed the second southern hemisphere cluster with principal component scores similar to *M. galloprovincialis* from the northern hemisphere, though once again there were some differences in allele frequencies, particularly at the *Mpi* and *Est-D* loci. Mussels from South Africa have similar allelic frequencies to *M. galloprovincialis* from the Mediterranean Sea and south-west England (Grant and Cherry, 1985; Beaumont *et al.*, 1989; Table 1).

B) MORPHOMETRIC CRITERIA: Overall shell morphology in *Mytilus* is subject to considerable phenotypic variation. Such environmental control of shape (and growth rate) is readily demonstrated by transplanting mussels from one habitat to another and recording the resulting changes in morphology [see Seed and Richardson (1990) and references therein]. Moreover, such environmentally induced variations are further confounded by ontogenetic changes in shape brought about by allometric growth (Seed, 1968, 1973, 1978, 1980). Similar trends are exhibited by both *Mytilus edulis* and *M. galloprovincialis* resulting in a considerable degree of convergence so that, in some populations, shell characters merge until identification on gross morphology alone becomes difficult or impossible. Intermixing of morphological characters in sympatric populations could also be due to hybridisation between these two mussels (e.g. Seed, 1972, 1974). In some populations, however, differences in gross shell morphology between *M. edulis* and *M. galloprovincialis* can be extremely pronounced. At Rock, for instance, where these two mussels occur in mixed populations, *M. galloprovincialis* has a significantly taller shell with a steeper ligamentary angle (the angle subtended by the ventral and ligamentary margins, see Fig. 2A) than *M. edulis*. Maximum shell width lies closer to the ventral margin and consequently the ventral aspect of *M. galloprovincialis* is much flatter when viewed in cross section (Figs. 2B, C). Typically *M. galloprovincialis* has a more pointed, beaked or ventrally incurved shell with a rather triangular shaped outline whereas *M. edulis* is more rounded anteriorly, has a more elongate, cylindrical shell with a straight or even slightly convex ventral margin. Thus, at Rock, several shell features combine within single individuals to produce mussels which are quite distinctive in their overall external appearance (Figs. 3H, I). Furthermore, these different morphologies are maintained amongst all size ranges of mussels strongly suggesting that they are, in fact, genetically rather than environmentally controlled (Beaumont *et al.*, 1989; Seed, 1990). Many of these features also recur in mussels from different parts of their geographical range (Figs.

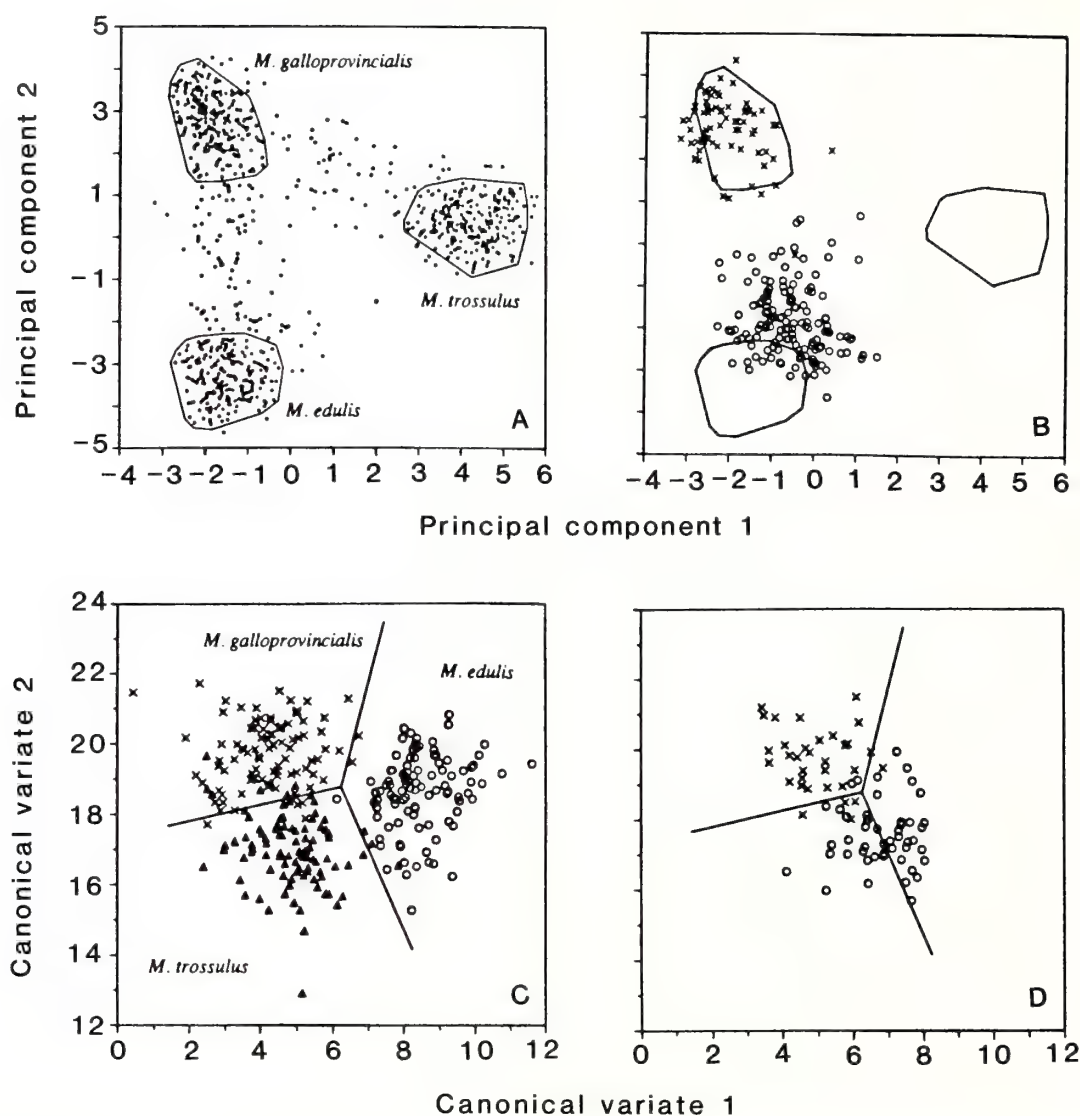


Fig. 1. First and second principal components of allozyme data for **A**, northern hemisphere, and **B**, southern hemisphere mussels. To aid visual comparison outlines have been drawn subjectively around the northern hemisphere clusters. **C**, **D**, First and second canonical variates of morphometric data for northern and southern hemisphere mussels respectively. Lines separating the northern hemisphere clusters have been drawn to aid comparison (after McDonald *et al.*, 1991).

3, 4).

In the mussel populations studied by Beaumont *et al.* (1989) the anterior adductor muscle to shell length ratios ($[aams/sl] \times 10$) in *Mytilus edulis* were consistently and significantly larger and the elongated scar more conspicuous than in its congener. The dark blue hinge plate in *M. edulis* is typically a more gently curved structure whereas in *M. galloprovincialis* it is usually paler in colour and describes a much tighter arc with the posterior end more closely delimited from the adjacent shell margin (Figs. 2B, C). Both the hinge plate to shell length ratio ($[hp/sl] \times 10$) and the length to width of the posterior byssal retractor scar ($lbrs/wbrs$) are significantly larger in *M. edulis*; in the latter ratio this is due almost entirely to variations in scar width rather than scar

length. On virtually all of the morphometric criteria used by Beaumont *et al.* (1989), *M. galloprovincialis* at Rock were statistically indistinguishable from conspecifics from Polzeath and South Africa. Frequency distributions of several of these morphometric characters are illustrated in figure 5 and show that whilst the mean values between these two mussels are markedly different, there is, nevertheless, a considerable degree of overlap in the ranges of these individual shell characters.

The value of the anterior adductor muscle scar and hinge plate as taxonomic characters for separating *Mytilus edulis* and *M. galloprovincialis* has been reported by several workers. In most of these studies (e.g. Lewis and Seed, 1969; Seed, 1978; Wilkins *et al.*, 1983; Grant and Cherry, 1985;

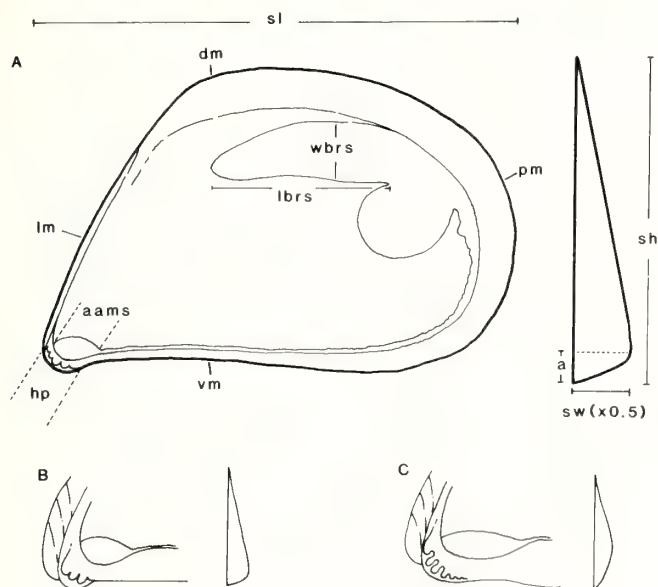


Fig. 2. A, Terminology of shell characters: a, position of maximum shell width along the dorso-ventral axis; aams, anterior adductor muscle scar; dm, dorsal margin; hp, hinge plate; lbrs, length of byssal retractor muscle scar; lm, ligamentary margin; pm, posterior margin; sh, shell height; sl, shell length; sw, shell width; vm, ventral margin; wbrs, width of byssal retractor muscle scar. Anterior end and transverse profiles of **B**, *Mytilus galloprovincialis* and **C**, *M. edulis* (after Beaumont *et al.*, 1989).

Lee and Morton, 1985) these characters have been considered separately but Verduin (1979) and Sanjuan *et al.* (1990) achieved a more effective separation when these were combined into a single taxonomic index. Other taxonomic characters previously used to separate these two mussels include the colour of the mantle edge, which is typically yellowish-brown in *M. edulis* and deep purple-violet in *M. galloprovincialis*, and the presence (*M. edulis*) or absence (*M. galloprovincialis*) of longitudinal rays of deeper colour in the shell (e.g. Hepper, 1957; Lewis and Seed, 1969).

By using four shell characters together with mantle edge colour and the genotypes of three enzyme loci (see p. 124) to identify *Mytilus edulis* and *M. galloprovincialis* Beaumont *et al.* (1989) were then able to test the reliability of each individual character against a final identification based on all eight characters. Table 2 shows the percentage of mussels that would have been misidentified using single taxonomic characters. The main point to emerge from this analysis was that no single character existed which allowed the certain identification of all mussels within these three populations. Overall, however, certain characters were clearly more reliable than others, though the diagnostic value of each character varied, sometimes quite markedly, both within and between sites. This applied equally to both morphometric and genetic characters. On average, single locus genotypes proved to be somewhat poorer diagnostic characters than the

polygenic morphometric characters though significant differences between populations were more easily detected by the electrophoretic than by the morphometric data (Beaumont *et al.*, 1989).

It is clear from the above that *individual* morphological characters in *Mytilus* can vary, often on an exceedingly localised scale, and are therefore of limited taxonomic value though certain characters, or combinations of characters, do permit the separation of these two mussels with a high degree of confidence at least in certain populations. Multivariate techniques, on the other hand, have proved to be more successful in discriminating between mussels within the *M. edulis* species complex. Using a canonical variates analysis of 19 different morphometric characters in those samples from northern hemisphere locations where allozyme analysis had indicated previously the presence of a single species, McDonald *et al.* (1991) were able to resolve three distinct clusters corresponding to *M. edulis*, *M. galloprovincialis* and *M. trossulus* (Fig. 1C). Somewhat surprisingly, the best discrimination was between *M. edulis* and *M. galloprovincialis*, a long standing taxonomic problem in this genus. Canonical variates analysis finds the linear functions of the morphological variables with coefficients that maximize the distance between groups that have been previously identified using some other criteria, in this case allozyme characters. When the functions from the canonical variates analysis of northern mussels were applied to southern hemisphere samples, southern *M. edulis* was found to be morphologically intermediate between northern *M. edulis* and *M. trossulus*; southern and northern *M. galloprovincialis*, by contrast, were remarkably similar to each other (Fig. 1D). Characters which have been considered previously useful for distinguishing *M. edulis* and *M. galloprovincialis*, such as the adductor muscle scar and hinge plate, also contributed most to the canonical variates analysis.

Thus, whilst some overlap occurred in the canonical variates, most individual mussels in these pure samples could be identified from shell characters alone when multivariate functions of all 19 morphometric variables were used. Individual characters, on the other hand, even those which are known to show the greatest variation between taxa, exhibited considerable overlap when these were considered singly. McDonald *et al.* (1991) also calculated canonical functions for each pair of northern mussels because all of the known areas of overlap between these mussels involve only two taxa. Results indicate that a linear combination of all characters in the canonical variate gives total separation in the case of *Mytilus edulis* and *M. galloprovincialis* (Fig. 6A) and an almost total separation of *M. edulis* and *M. trossulus* (Fig. 6B). For *M. galloprovincialis* and *M. trossulus*, which share several morphological traits, particularly with regard to their overall shell shape (Figs. 3, 4) and small size of the anterior adductor scars and hinge plates, there was a somewhat greater

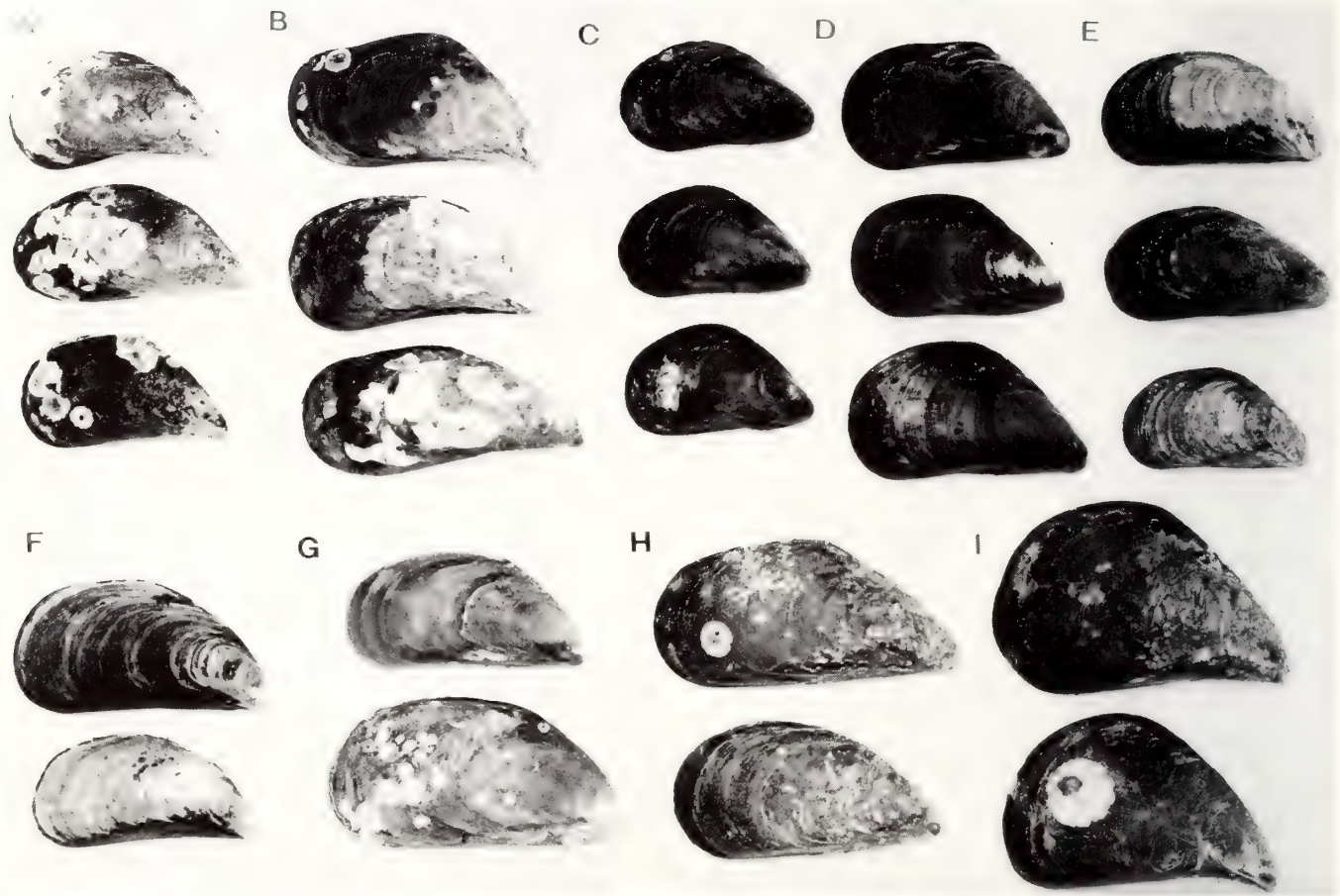


Fig. 3. *Mytilus trossulus* from: A, Tillamook, Oregon; B, Newport, Oregon. *M. edulis* from: C, Stony Brook, New York; D, Portland, Maine; E, Aarhus, Denmark; F, Falkland Islands; G, Mar del Plata, Argentina; H, Rock, S. W. England and *M. galloprovincialis* from: I, Rock, S. W. England (scale bar in cm).

degree of overlap (Fig. 6C). The posterior byssal retractor scar of *M. trossulus*, however, is characteristically much narrower than that of *M. galloprovincialis* of comparable shell length. Further research is now required to determine whether the morphometric differences described by McDonald *et al.* (1991) for pure mussel samples persist in areas of overlap and hybridisation. Moreover, by incorporating additional morphological characters into the multivariate analysis it would seem likely that an even better discrimination of these mussels could be achieved.

C) OTHER CRITERIA: Quite apart from the genetic and morphometric differences described above, *Mytilus edulis* and *M. galloprovincialis* are also known to vary in several other important respects. Figure 7 shows that, at Rock, spawning in *M. edulis* occurs mainly during May and June whereas *M. galloprovincialis* does not spawn until late July or August when seawater temperatures for this geographical locality are maximal. The cyclical pattern of reproduction is also less pronounced in *M. galloprovincialis* with significant proportions

of fully ripe individuals persisting throughout much of the year. These differences are documented in detail elsewhere (Seed, 1971) but, in summary, extensive hybridisation at this particular site in south-west England seems most unlikely, a conclusion which is broadly supported by electrophoretic data (e.g. Skibinski *et al.*, 1983; Beaumont *et al.*, 1989). Temporal differences in spawning activity between these mussels have been reported similarly at another site in south-west England (Croyde) where *M. galloprovincialis* also had a greater estimated annual fecundity than *M. edulis* (Gardner and Skibinski, 1990); at a second site (Whitesand), however, there was a higher degree of genetic mixing resulting from reduced levels of variability in the timing of spawning and fecundity (Fig. 8).

Hybridisation can be induced artificially in the laboratory and when *Mytilus edulis* and *M. galloprovincialis* are crossed they produce fertile hybrids which can then backcross to the parent form to produce viable offspring (Lubet *et al.*, 1984). There would appear to be little evidence, therefore, of any *absolute* reproductive barrier or genetic in-



Fig. 4. *Mytilus galloprovincialis* from: **A**, Los Angeles, California; **B**, Victoria Harbour, Hong Kong; **C**, Albany, Western Australia; **D**, Huon River Estuary, Tasmania; **E**, Venice, Italy; **F**, West Cape coast, S. Africa; **G**, Newquay, S. W. England; **H**, Polzeath, S. W. England; **I**, Parede, Portugal; **J**, Vigo, Spain (scale bar in cm).

compatibility between these two mussels. However, whilst Lubet *et al.* (1984) apparently were unable to detect any adverse effects on viability, growth or mortality amongst the F^1 hybrids, recent research has shown that the mortality rates of hybrid larvae can be substantially higher than those of pure *M. edulis* or pure *M. galloprovincialis* larvae (Table 3).

Mytilus edulis and *M. galloprovincialis* can exhibit markedly different levels of infection by certain parasitic organisms (e.g. Seed, 1969, 1978; Coustau *et al.*, 1990; Hillman, 1990). Such differences appear to have a genetic rather than an ecological basis, and because these parasites can influence fitness through their effects on fecundity and condition, they could provide a potentially important selective force in sympatric mussel populations. Table 4 shows that on average approximately 30% of the *M. edulis* population at Rock is infested by the peacrab *Pinnotheres pisum* Penn. whereas in *M. galloprovincialis* the level of infestation is less than 2%. Several immunological (e.g. Bisignano *et al.*, 1980; Brock, 1985), histopathological (e.g. Hillman,

1990) and chromosomal (e.g. Thiriot-Quievreux, 1984; Dixon and Flavel, 1986; Pasantes *et al.*, 1990) investigations are available for *Mytilus*, albeit with somewhat equivocal results. Significant differences in sperm size and morphology also have been described (e.g. Drozdov and Reunov, 1986; Hodgson and Bernard, 1986; Crespo *et al.*, 1990). Whilst the cytological differences reported within this genus are clearly insufficient to prevent hybridisation, they could, nonetheless, be partially responsible for maintaining species separation and could also presumably serve as useful taxonomic characters.

Only recently has the analysis of mitochondrial DNA variation been used in taxonomic studies of marine mussels (e.g. Skibinski, 1985; Blot *et al.*, 1990). Several pure and mixed populations of *Mytilus edulis* and *M. galloprovincialis* have been studied (e.g. Edwards and Skibinski, 1987; Fisher and Skibinski, 1990) and, whilst significantly different mtDNA genotypes were reported, none was perfectly diagnostic. There is little evidence, therefore, to suggest that

Table 2. Percentage of mussels which would have been misidentified using single taxonomic characters (from Beaumont *et al.*, 1989).

	n	Overall shape	aams ¹	hp ²	Mantle colour	Raying	Mpi	Est-D	Odh
i) Rock:									
<i>Mytilus edulis</i>	64	0	0	4.7	0	17.2	23.8	3.2	25.0
<i>M. galloprovincialis</i>	76	34.2	17.1	10.5	6.6	1.3	7.9	9.4	36.5
ii) S. Africa:									
<i>M. galloprovincialis</i>	38	23.7	5.3	10.5	0	0	21.1	28.9	21.1
iii) Polzeath:									
<i>M. edulis</i>	3	0	0	0	0	33.3	66.6	0	33.3
<i>M. galloprovincialis</i>	81	11.3	19.4	9.7	0	3.2	48.4	88.7	32.3
Mean		16.8	11.8	8.8	1.9	6.1	26.7	35.1	29.8

^{1,2} Anterior adductor muscle scar and hinge plate, respectively.

mtDNA variation provides any greater overall diagnostic power than allozyme variation in distinguishing between the different forms of *Mytilus* though mtDNA studies should ultimately lead to an improved understanding of both the population biology and taxonomy of this genus (Edwards and Skibinski, 1987).

ORIGINS AND DISTRIBUTION

With a geological record extending back for less than two million years, the genus *Mytilus* is of relatively recent origin (Seed, 1976). Amongst the smooth-shelled taxa, *M. edulis* generally is considered to be the ancestral species ap-

parently having evolved from some more primitive infaunal or semi-infaunal modiolid stock (Stanley, 1972; Seed, 1990). *M. edulis* is widely distributed throughout the temperate latitudes of both hemispheres. In the northern hemisphere it occurs along the eastern seaboard of North America as far south as Cape Hatteras in North Carolina, but evidence now suggests that this species is absent from the Pacific coast of the north American continent (McDonald and Koehn, 1988). In Europe it extends from the Arctic waters of the White Sea and northern Norway southwards to north Africa (Seed, 1976; Suchanek, 1985) although recent work by Sanjuan *et al.* (1990) suggests that mussels along the whole of the Iberian peninsula could in fact be *M. galloprovincialis*, and that the

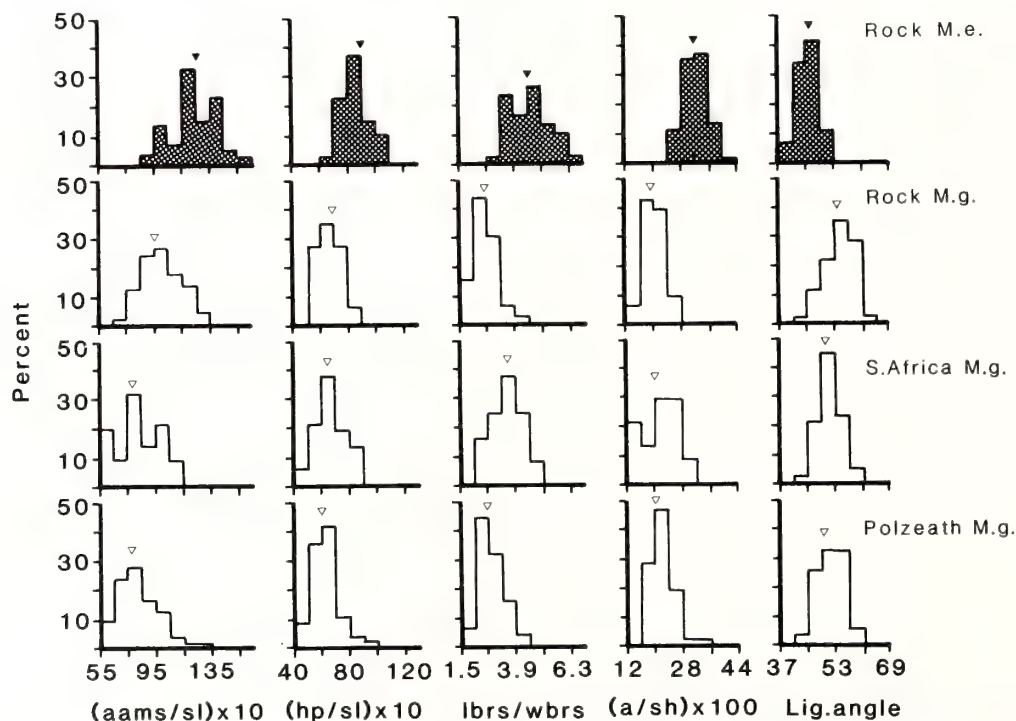


Fig. 5. Frequency distributions of several morphometric characters in *Mytilus edulis* and *M. galloprovincialis*. Mean values denoted by arrowheads. Note the similarity between the three *M. galloprovincialis* samples and how these differ from *M. edulis* (for abbreviations see figure 2) (after Beaumont *et al.*, 1989).

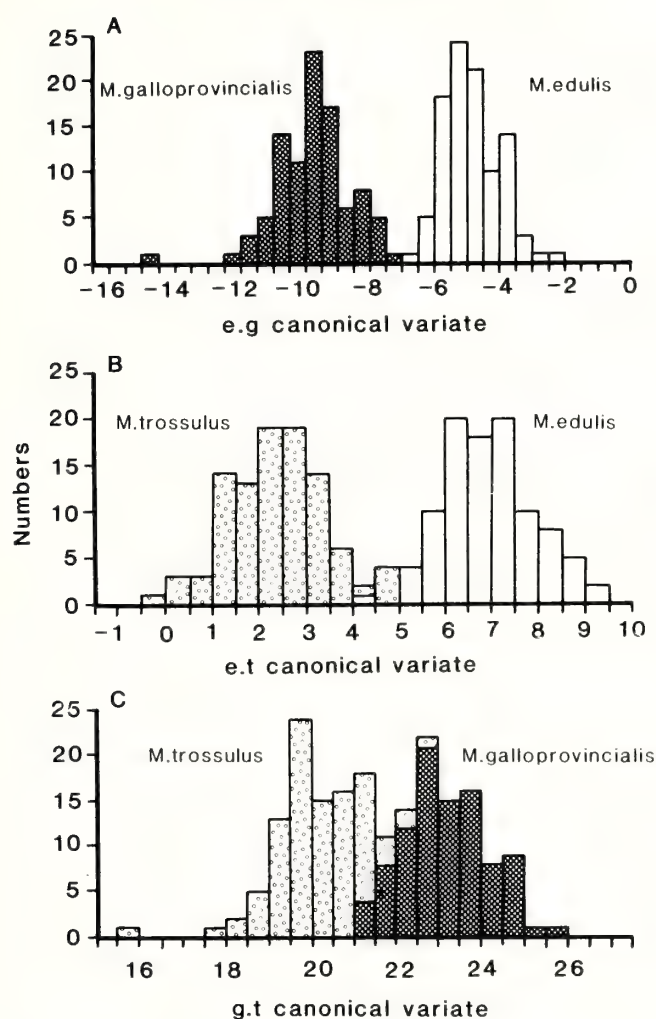


Fig. 6. Distribution of canonical variates for pairs of species from the northern hemisphere (after McDonald *et al.*, 1991).

southern limit of *M. edulis* is probably further north than was suspected previously. It is present in Iceland (Varvio *et al.*, 1988) and in Hudson Bay (Koehn, 1991), but its occurrence in Greenland, Novaya Zemlya and along the Arctic coast of Canada is still in question. In the southern hemisphere *M. edulis* occurs in the Falkland islands and along the east and west coasts of South America (as *M. platensis* and *M. chilensis* respectively). Mussels from the Kerguelen islands (= *M. desolationis*) are tentatively regarded as *M. edulis* (McDonald *et al.*, 1991).

Mytilus galloprovincialis also occurs in temperate waters of both hemispheres but its range extends into much warmer latitudes than *M. edulis*. This mussel is thought to have evolved from the *M. edulis* stocks which were present originally both on the Atlantic and Mediterranean coasts (Barsotti and Meluzzi, 1968). The warmer conditions which developed in the Mediterranean and the reduced contact be-

tween the Mediterranean and Atlantic during one of the Pleistocene ice ages favoured the differentiation of these stocks - a process which is probably still in progress (Seed, 1978). Recent studies on mtDNA suggest a divergence time between *M. edulis* and *M. galloprovincialis* which is consistent with palaeontological evidence (Fisher and Skibinski, 1990). Northerly migration of *M. galloprovincialis* probably occurred as the ice cap retreated, and in Europe this mussel is now present along much of the Atlantic coasts of Britain, France and Ireland where it coexists and hybridises to varying degrees with *M. edulis* (e.g. Seed, 1978; Gosling and Wilkins, 1981; Skibinski *et al.*, 1983).

M. galloprovincialis has also been introduced to areas far removed from its region of origin and in each case the introduced population is strikingly similar, both genetically and morphologically, to Mediterranean populations of this mussel. In the northern hemisphere its presence has been confirmed in California (McDonald and Koehn, 1988), Japan (Wilkins *et al.*, 1983), Hong Kong (Lee and Morton, 1985) and along the east China coast northwards as far as the border between Korea and the Soviet Union (McDonald *et al.*, 1991). These introductions were probably relatively recent events though *M. galloprovincialis* could have been present in California (as *M. diegensis*) since the turn of the century (McDonald and Koehn, 1988). In the southern hemisphere it occurs in South Africa (Grant and Cherry, 1985) and is widely distributed (as *M. planulatus*) throughout Australasia (McDonald *et al.*, 1991); its absence from South America is intriguing given the long history of trading between this Continent and countries bordering the Mediterranean.

Different allele frequencies between southern and northern populations of *Mytilus* have led to speculation that many southern mussel populations (of both *M. edulis* and *M. galloprovincialis*) could be native rather than introduced. Support for this view is provided by the occurrence of *Mytilus*-like fossils or subfossils in Australasia (Fleming, 1959; Don-

Table 3. Summary of laboratory fertilisation and larval survival experiments (Beaumont, Matin and Seed, unpub.).

Treatment ¹	Survival (%) after		Abnormal
	3 days ²	9 days ³	larvae (%)
pure lines:			
e/e; g/g	69	70	44
	ns	**	ns
hybrids:			
e/e; g/e	62	37	38

¹Each treatment consisted of 12 replicates.

²All replicates started with 50 or 100 x 10³ eggs.

³Cultures maintained at constant larval densities (numbers.ml⁻¹) by adjusting volume.

**p < 0.01; ns, not significant

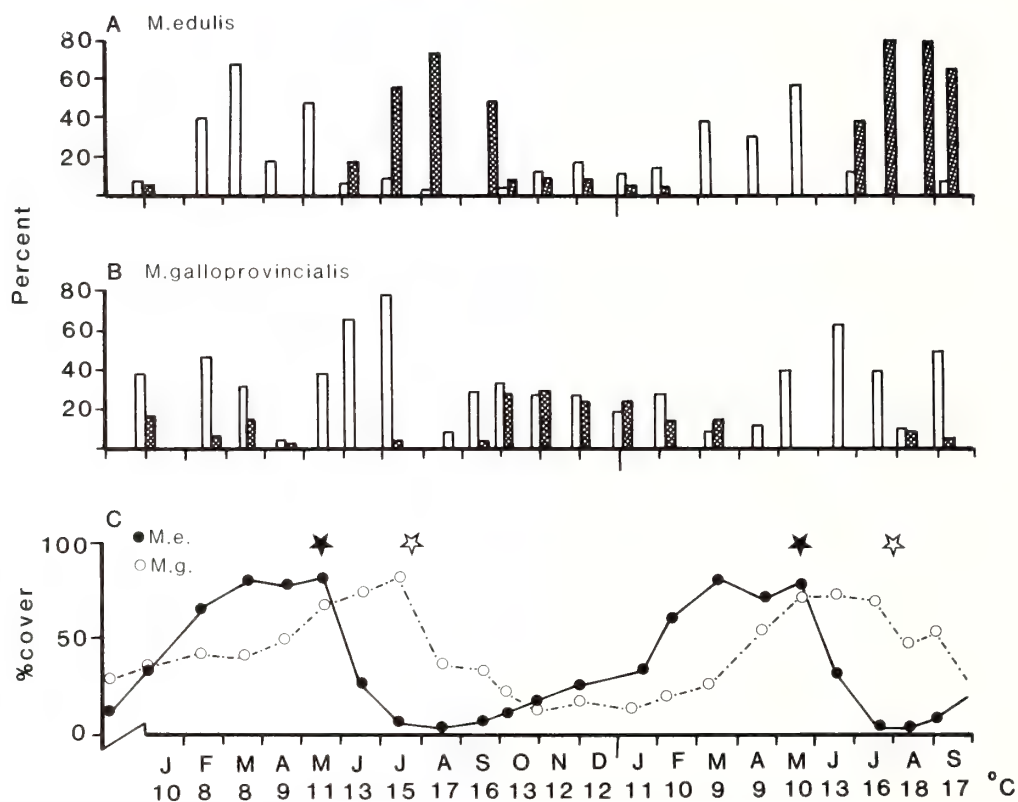


Fig. 7. Reproductive cycles of **A)** *Mytilus edulis* and **B)** *M. galloprovincialis* at Rock, S. W. England. Open columns denote ripe individuals, stippled columns spent individuals **C)** Area occupied by reproductive follicles in histological sections of mantle tissue; asterisks indicate onset of the main spawning periods (after Seed, 1971).

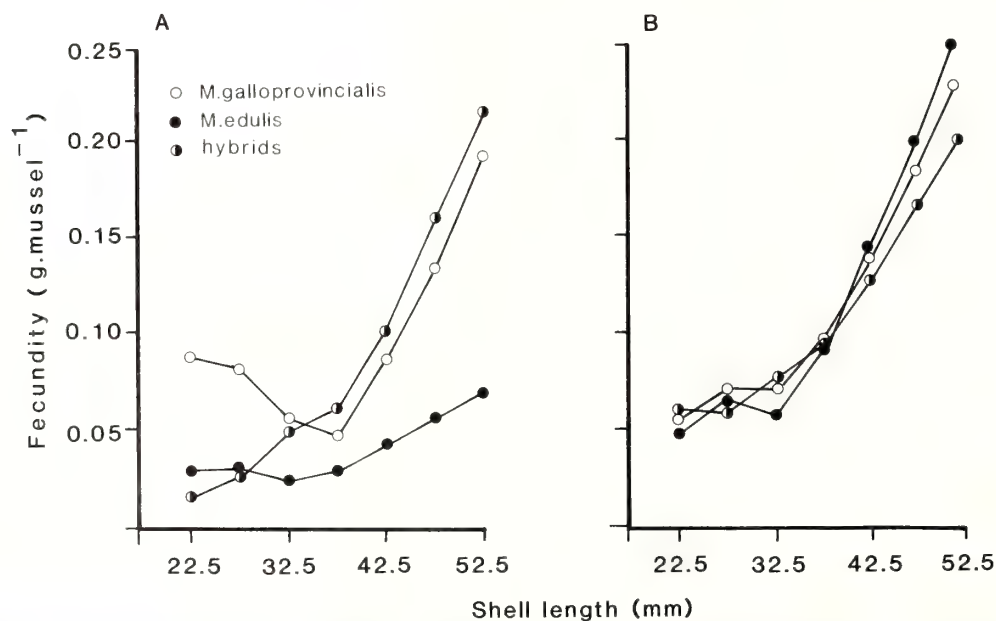


Fig. 8. Total annual fecundity as a function of genotype and shell length in mussels from **A)** Croyde and **B)** Whitsand, S. W. England (after Gardner and Skibinski, 1990).

Table 4. Incidence of *Pinnotheres pisum* and the proportions of *Mytilus edulis* and *M. galloprovincialis* in low shore mussels at Rock¹.

Date	<i>M. edulis</i>	<i>M. galloprovincialis</i>	Proportion (%)	
	% infected (n)	% infected (n)	M.e.	M.g.
1. 1952 ²	—	—	15	85
2. Nov 1966; Jan 1968	30.5 (128)	4.5 (112)	—	—
3. Jun 1968	45.3 (316)	2.8 (212)	16	84
4. May 1968-Aug 1969	30.1 (718)	1.4 (768)	—	—
5. Oct 1985 ³	22.6 (230)	0 (148)	14	86
6. Oct 1989	—	—	17	83
Mean (total)	32.3 (1392)	1.8 (1240)	15.5	84.5

¹All mussels exceeded the min. length (3.35cm) at which infection occurs.²From Hepper (1957).³Larger more heavily infected mussels less abundant than in earlier collections.

ner and Jungner, 1981; Kerrison and Binns, 1984) and South America (Johnson, 1976). The possibility still remains, however, that native species of *Mytilus* could have interbred subsequently with, or been largely displaced by, introduced mussels of northern origin. The absence of *Mytilus* from aboriginal shell middens and raised-beach deposits in South Africa and from early museum collections in Japan and South Africa (Wilkins *et al.*, 1983; Grant and Cherry, 1985) is consistent with the view that the present populations of *M. galloprovincialis* were introduced. Because *M. galloprovincialis* is widespread in the South Pacific, introductions into the northern Pacific need not, however, have originated in Europe (Koehn, 1991) though the genetic similarity between what are believed to be introduced populations and Mediterranean *M. galloprovincialis* would tend to argue against this view.

Mytilus trossulus has a rather disjunct distribution occurring in the colder waters along both sides of the Atlantic and Pacific oceans. It is present on the west coast of North America from central California to Alaska (McDonald and Koehn, 1988), along the Pacific coast of the Soviet Union (McDonald *et al.*, 1991), in the Maritime Provinces of north-eastern Canada (Koehn *et al.*, 1984) and in the Baltic Sea (Varvio *et al.*, 1988; Bulnheim and Gosling, 1988). Varvio *et al.* (1988) have suggested a relatively ancient (1-2myr) northern origin for this lineage which probably evolved from some cold tolerant genotype during the Pleistocene glacial period; this could explain why its present distribution is broadly confined to regions just south of areas that were previously ice covered. Koehn (1991) argues that *M. trossulus* could in fact be a zoogeographical remnant of what was once a far more widely distributed mussel. To date, *M. trossulus* has not been recorded in the southern hemisphere.

Mytilus californianus is restricted to the Pacific coast of North America where it ranges from the Aleutian islands in Alaska to northern Mexico (Seed, 1976). *M. coruscus* occurs in Japan, and on the Pacific coast of Asia in China, Korea and Siberia (Scarlato, 1981). The geographical ranges of these

two mussels, therefore, overlap with those of *M. trossulus* and *M. galloprovincialis* though *M. californianus* and *M. coruscus* are fairly easily differentiated from these smooth-shelled mussels on shell characteristics alone.

The global distribution of *Mytilus*, based largely on the extensive survey by McDonald *et al.* (1991), is illustrated in figure 9. This survey, however, was not intended to include the small scale sampling which will clearly be required in order to establish the precise geographical and ecological ranges of the various taxa, as well as the extent of hybridisation. The small scale variations in distribution are well illustrated by reference to one of the sites studied by McDonald *et al.* (1991), Posjet Bay in the Soviet Union, where mussels from an intertidal beach contained only *M. trossulus* whilst mussels from a floating dock just a few meters away were all *M. galloprovincialis*. At sites in Britain and Ireland, where *M. edulis* and *M. galloprovincialis* coexist, *M. galloprovincialis* often predominates on wave exposed shores, particularly at higher tidal elevations, whereas protected bays and estuaries are more typically favoured by *M. edulis* (e.g. Gosling and Wilkins, 1977, 1981; Skibinski *et al.*, 1983; Skibinski and Roderick, 1991; Gosling and McGrath, 1990). *M. galloprovincialis* is known to have stronger byssal attachment than *M. edulis* (Gardner and Skibinski, 1990, 1991) and also possesses shell features that enhance physical stability on hard surfaces (Seed, 1978, 1990). Such attributes could explain the apparent success of this mussel in high energy environments. It is interesting to note, therefore, that *M. californianus*, which shares several features with *M. galloprovincialis* (e.g. shell shape, strong byssal attachment) also predominates on wave exposed shores (Harger, 1972; Seed and Suchanek, 1992).

TAXONOMIC RELATIONSHIPS

Mytilus taxonomy has relied traditionally on morphological shell characters but these are greatly influenced by environment, and their diagnostic value is therefore often questionable. Enzyme electrophoresis, restriction analysis of

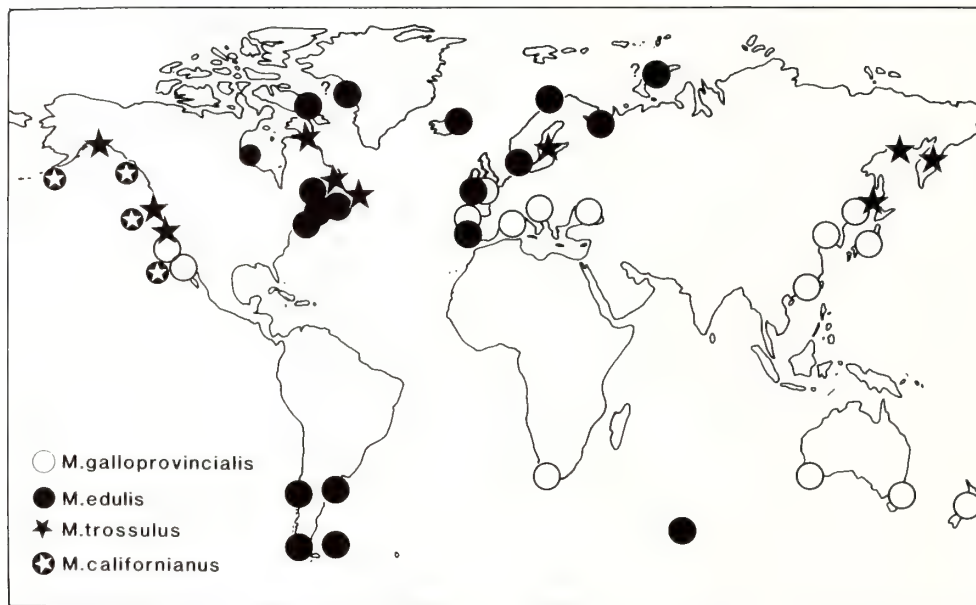


Fig. 9. Global distribution of the three smooth-shelled mussels, *Mytilus edulis*, *M. galloprovincialis* and *M. trossulus* (mainly from McDonald *et al.*, 1991). The distribution of *M. californianus* is also shown.

mtDNA and amino-acid sequencing are relatively free of environmentally induced changes and these techniques, together with immunological and cytological studies are now playing an increasingly important role in the systematic characterisation of *Mytilus* worldwide. At present, no single character exists which separates the three smooth-shelled taxa, *M. edulis*, *M. galloprovincialis* and *M. trossulus* unequivocally, though certain characters and combinations of characters are clearly more diagnostic than others. Recently, McDonald *et al.* (1991) effectively discriminated among these taxa using a multivariate approach and in all cases analyses of allozyme and morphometric data gave concordant results. Furthermore, mtDNA sequence variation data are so far broadly consistent with the taxonomic judgements based on both allozyme and morphometric data (Koehn, 1991). Such studies serve to emphasise the value of a multidisciplinary approach in resolving complex taxonomic problems.

Whether *Mytilus edulis*, *M. galloprovincialis* and *M. trossulus* should be considered as separate species has been the focus of considerable discussion (e.g. Gosling, 1984; Skibinski *et al.*, 1983; Blot *et al.*, 1988; Bulnheim and Gosling, 1988; Johannesson *et al.* 1990; Väinölä, 1990). One reason for the reluctance to consider these taxa as distinct species is that in areas of geographical overlap allozyme characters indicate varying degrees of hybridisation and introgression (e.g. Skibinski and Beardmore, 1979; Gosling and Wilkins, 1981; Skibinski *et al.*, 1983; McDonald and Koehn, 1988; Koehn, 1991; Väinölä and Hvilsom, 1991) with the concomitant mixing of morphological characters (e.g. Seed, 1972, 1974). Unfortunately, there is no generally accepted

maximum amount of hybridisation which two taxa can exhibit and still be considered separate species. Hybrid zones of these mussels vary in size and are spatially complex with pure, mixed and hybrid populations occurring in a patchwork pattern. The most geographically widespread hybridisation occurs between *M. edulis* and *M. galloprovincialis* existing from the Biscay coast of France or even northern Spain to parts of northern Britain. Hybridisation between North Sea *M. edulis* and Baltic *M. trossulus*, by contrast, occurs over a relatively narrow zone in the Danish Belt Sea. Contact between *M. edulis* and *M. trossulus* in North America is poorly documented, but from the available evidence hybridisation occurs at several sites in the upper reaches of the Gulf of St. Lawrence. In central California *M. galloprovincialis*, *M. trossulus* and their hybrids are present. No hybridisation has so far been reported in the region near the border between Korea and the Soviet Union where there is contact between *M. trossulus* and *M. galloprovincialis*, but this could simply reflect the lack of detailed information for this particular geographical area.

Hybrid zones between these mussels also appear to be relatively stable indicating that although gene flow does occur, the parent forms can still maintain their genetic (and morphological) integrity. In south-west England the proportions of *Mytilus edulis* and *M. galloprovincialis* have remained virtually unchanged over a period of almost 40 years (Table 4) despite the occurrence of hybridisation. Moreover, these proportions are virtually identical amongst all size categories of mussels (but see Gardner and Skibinski, 1990). We have no clear evidence therefore that *M. galloprovincialis* in this

particular geographical locality is gradually replacing *M. edulis*; this is perhaps surprising in view of the higher fecundity and competitive edge that *M. galloprovincialis* apparently enjoys over its congener (e.g. Gardner and Skibinski, 1988, 1990, 1991; Skibinski and Roderick, 1991). If directional selection in favour of *M. galloprovincialis* is occurring then it is obviously being offset by the immigration of *M. edulis* from other localities. Current genetic and morphometric data suggest that gene flow between *M. edulis* and *M. galloprovincialis* in the Rock population is limited. This of course partly reflects the different reproductive cycles of these two mussels at this site (Fig. 7) although in laboratory experiments we now know that hybrid larvae can experience heavy mortality rates (Table 3) thus presumably contributing to the temporal genetic stability in sympatric populations of these two mussels (see also Gardner and Skibinski, 1988; Gosling and McGrath, 1990). A considerable amount of selection against hybrid individuals could therefore conceivably occur *before* juvenile mussels are actually recruited to the established population.

Despite the lack of any absolute reproductive barrier and the massive potential for dispersal via a planktonic larval stage that can last for several weeks, populations of *Mytilus edulis*, *M. galloprovincialis* and *M. trossulus* comprise relatively homogenous groups each maintaining a unique genetic and morphological phenotype across vast distances. This distinctiveness warrants recognition and it is perhaps appropriate therefore to consider these taxa as three distinct species despite the occurrence of localised hybridisation (McDonald *et al.*, 1991). Differences in mtDNA fragments, sperm size and structure, as well as chromosomal variations (p. 129) further support the taxonomic interpretation that the genetic differences between these mussels are quite substantial and that they ought therefore to be accorded separate and equal systematic status. In an earlier paper (Seed, 1978) I have argued that *M. galloprovincialis* could be an emerging species, reaching specific status in certain parts of its geographical range whilst freely interbreeding elsewhere. Far from straining the biological species concept this merely emphasises the problems inherent in extending the concept geographically. Tentative synonymies of *Mytilus* are summarised in Table 5.

It is clear from the *Mytilus galloprovincialis* controversy that a multidisciplinary approach is required if the complex systematics of the genus *Mytilus* are to be satisfactorily resolved. In addition to further research using allozyme and morphometric characters, particularly applied to the hitherto poorly studied populations in the southern hemisphere, promising areas for future work include: 1) studies of reproductive cycles and fecundity in sympatric populations; 2) measurements of survival, growth and physiological parameters in natural and laboratory hybrids; 3) studies of abnormal development, growth and survival in pure and

Table 5. Simplified and tentative synonymies of *Mytilus* spp.

i) <i>M. edulis</i> Linnaeus	(= <i>M. platensis</i> Orbigny <i>M. chilensis</i> Hupé <i>M. desolationis</i> Lamy = <i>M. kerguelensis</i> Fletcher)
ii) <i>M. galloprovincialis</i> Lamarck	(= <i>M. diegensis</i> Coe <i>M. planulatus</i> Lamarck <i>M. aoteanus</i> Powell <i>M. zhirmunskii</i> Scarlato and Starabogatov)
iii) <i>M. trossulus</i> Gould	(= <i>M. kussakini</i> Scarlato and Starabogatov)
iv) <i>M. californianus</i> Conrad	
v) <i>M. coruscus</i> Gould	[= <i>M. crassitesta</i> Lischke (= <i>M. californianus</i> ?)]

hybrid larvae; 4) reciprocal transplants of mussels between the ranges of the different taxa; 5) comparisons of sperm morphology and an extension of the mtDNA, karyological and immunological studies of mussels from pure and hybrid populations. The aim of this research should not be to determine once and for all whether these *Mytilus* taxa are 'good species'. This is unresolvable without an agreed operational definition of a biological species and in any case is perhaps a somewhat semantic question largely devoid of biological interest. Instead, future research should concentrate on the biological processes which keep these taxa distinct despite the widespread occurrence of hybridisation.

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Date of manuscript acceptance: 11 December 1991

The Australasian Protocardiinae revisited (Bivalvia: Cardiidae)

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Abstract. The Protocardiinae, the oldest unquestionable group of the family Cardiidae, has a long geological existence in Australasia. Six Recent Australasian species have been recognized and are traditionally referred to *Nemocardium* s.l. Two of them belong to *Pratulium*, a genus-group now restricted to relatively deep biotopes in warm temperate waters of Australasia. Considering its characteristic sculptural pattern and restricted Australian-New Zealand distribution since the Cretaceous, *Pratulium* is considered here to be a distinctive genus. The genera *Microcardium* and *Trifaricardium* are reported for the first time from Australasia. The other Australian species of Protocardiinae are classified in *Lyrocardium* and *Frigidocardium*, with a single species remaining in *Nemocardium* s.s.

Since two new species of *Microcardium* were obtained in the upper bathyal zone by the French oceanographic expedition MUSORSTOM-I in the Philippine Islands (Poutiers, 1981), further investigations allowed the accumulation of new data on Recent and fossil Cardiidae. This has rendered necessary the publication of a series of papers devoted to this family, and specially to members of the subfamily Protocardiinae. This paper is intended only as a preliminary note on the subject, in which the present author gives some results of his studies on Australasian Protocardiines, and discusses the taxonomy, biogeography and paleontology of the group.

This note is based on an extensive suite of fossil and Recent material from the Indo-Pacific area, including material collected since 1977 during several oceanographic expeditions organised by the Paris Muséum national d'Histoire naturelle (Richer de Forges, 1990) as well as historic and recently collected material obtained from various institutions. Detailed analysis of the material used in this study, and descriptions of new taxa illustrated here will appear in subsequent publications.

Since the subfamily Protocardiinae was established (Keen, 1951), there have been only a few studies on this group as a whole (Keen, 1969, 1980; Habe, 1977; Kafanov and Popov, 1977; Popov, 1977). To date, most authors have adopted the taxonomic scheme advanced by Keen.

FOSSIL RECORD

The protocardiines are the oldest unquestionable representatives of the Cardiidae. Their fossil record extends back to the Upper Triassic and they diversified largely during the Mesozoic. This is especially true for *Protocardia* Beyrich, 1845, the type-genus of the subfamily, which is known from Upper Triassic to Upper Cretaceous. Only one

other genus, *Septocardia* Hall and Whitefield, 1877, allegedly belonging to the subfamily Cardiinae, occurs in the Upper Triassic, but its placement in the Cardiidae has not received a general acceptance among taxonomists (Kafanov and Popov, 1977). Two taxa, *Nemocardium* Meek, 1876, and *Pratulium* Iredale, 1924, bridged the gap between Mesozoic and Cenozoic (Keen, 1950, 1980; Skwarko, 1983), and they still occur in the Recent fauna. They are the only members of the family to show this stratigraphic distribution. After a second period of radiative evolution during the Tertiary, Protocardiines nowadays survive mainly on the outer shelf and upper continental slope. This is a rather deep-water habitat for the family that occurs typically in the shallow waters of coastal to subtidal environments. Thus, in a way, Recent Protocardiines can be considered as a relict deep-water group within the family Cardiidae.

BIOGEOGRAPHY

The subfamily Protocardiinae is present in nearly every tropical region, and the western Pacific Ocean is the area of maximum species diversity with 15 of the 26 known species worldwide (Fig. 1). The genus *Microcardium* Thiele, 1934, is a good example. No less than three species are known from Philippine waters, two of which have been described recently (Poutiers, 1981). The most common of these, *Microcardium aequiliratum* Poutiers, 1981 (Fig. 2a), lives in fine soft bottoms at depths of 180m to 350m. In addition to the Western Pacific Ocean, Protocardiines occur also in the Indian Ocean (six species), Eastern Pacific (five species), as well as in the Western and Eastern Atlantic Ocean (three and one species respectively).

In Australasia, five Recent species of Protocardiines are known from Australia (two of which occur also in New

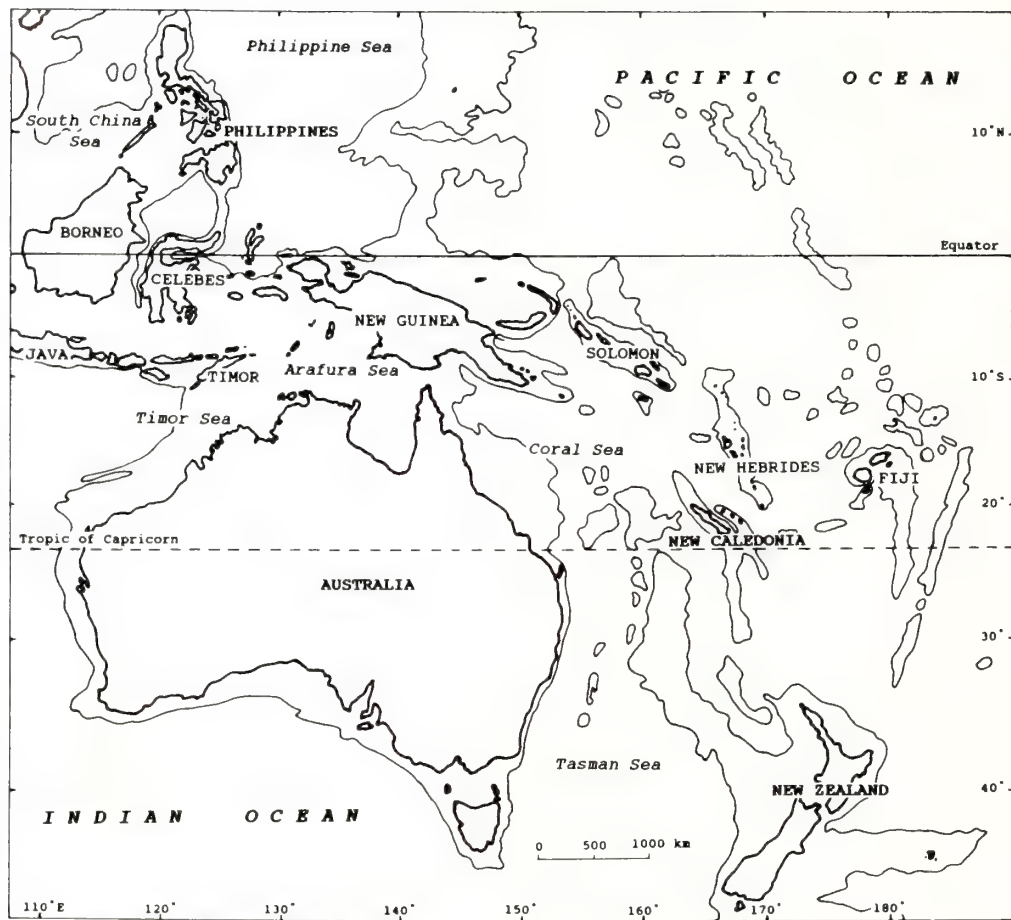


Fig. 1. Simplified map of Australasia and neighbouring areas.

Caledonia), and one from New Zealand. They are traditionally allocated to the broadly defined genus *Nemocardium*, as are all Recent species of the Protocardiinae (Keen, 1969, 1980). This placement seems to be supported by the rather conservative aspect of conchological characters in the subfamily, and by a frequent occurrence of secondary convergence that makes the differentiation of lineages sometimes problematical.

According to the climatic zonation of the world oceans, two main biogeographical areas are recognized currently in Australasia. In these areas, a number of faunal regions and provinces have been defined for the intertidal and shelf zones, on the basis of distribution and the degree of endemism of the benthic and demersal organisms (Wilson, 1971; Briggs, 1974; Pielou, 1979). Thus, northern Australia, New Caledonia and its dependencies (Fig. 1) are included in the tropical Indo-West Pacific Region, whereas southern Australia and northern New Zealand correspond to a warm temperate area forming the Southern Australian and the Northern New Zealand Regions.

This biogeographical pattern is reflected in the distribution of Protocardiinae in Australian waters. *Nemocardium* is

present in the tropical region, represented by *N. bechei* (Reeve, 1840), the only living species of *Nemocardium* s.s. (Fig. 2b). *Pratulium* occurs in the temperate region, represented by *P. thetidis* (Hedley, 1902), type-species of the genus (Fig. 2c). *P. thetidis* was thought by Hedley (1902) to be only a local variety of *Cardium striatum* Sowerby, 1834, an older but incorrect name for the New Zealand species *P. pulchellum* (Gray, 1843) (Fig. 2d). However, Iredale (1924) correctly distinguished these species when he erected *Pratulium*.

Pratulium appears to be confined nowadays to the temperate waters of Australasia. The only record of *Pratulium* from tropical Australasia is that of Fischer-Piette (1977). This seems to be an error based on the incorrect labelling of an old specimen, and I have been unable to find any member of this genus in the large samples collected recently in that region by the Paris Muséum national d'Histoire naturelle. However, paleontologists often consider *Pratulium* a Tethyan Indo-Pacific element, like *Nemocardium* s.s. For example, Darragh (1985), using a similar approach to Fleming (1962, 1967), analysed the composition of the molluscan fauna to define a tentative biozonation of the Tertiary in southeastern Australia and included *Pratulium* under the Tethyan Indo-

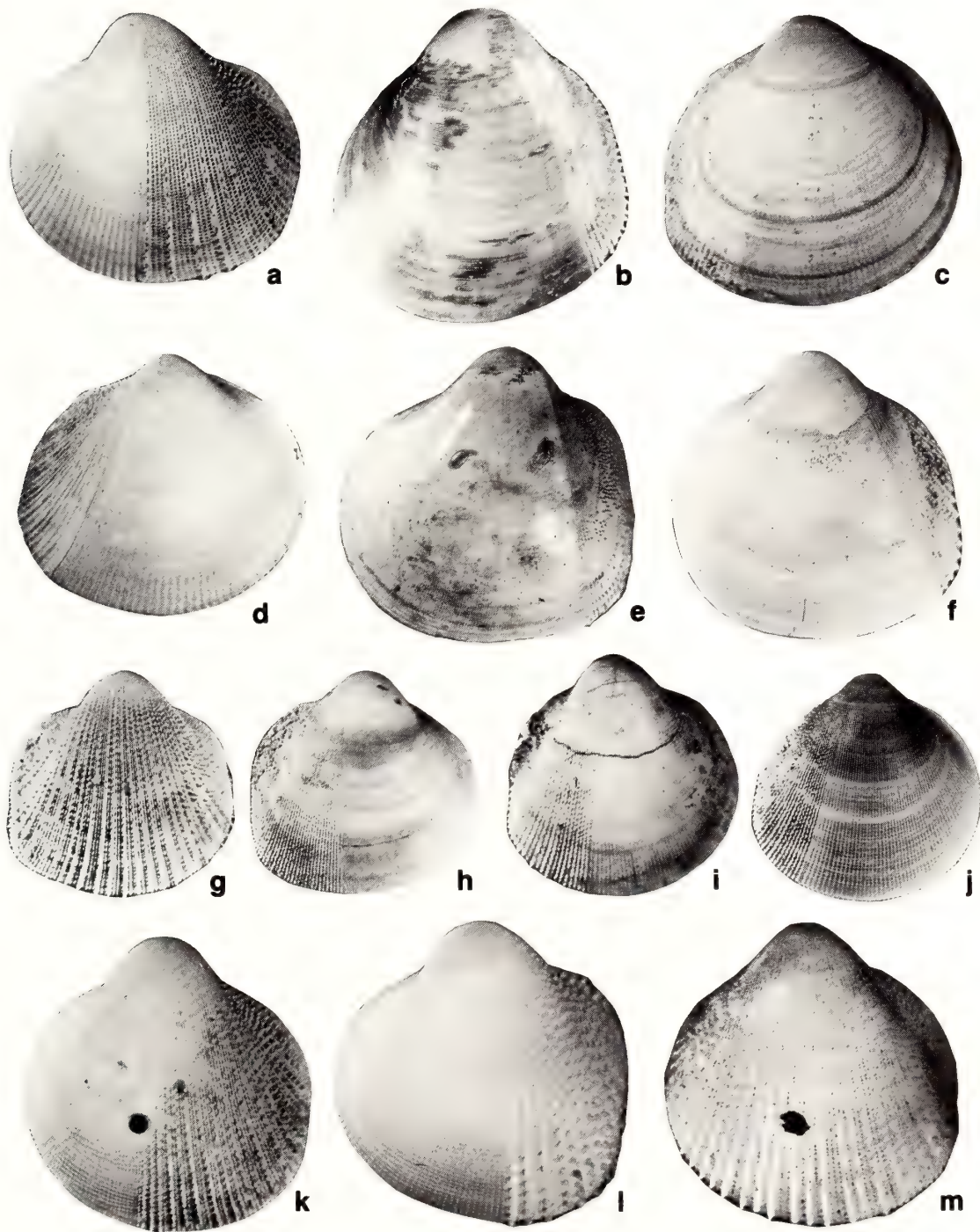


Fig. 2. External views of Recent and Tertiary species of Protocardiinae, showing diversity of sculpturing among genera (AMS, Australia Museum, Sydney; MNHN, Muséum national d'Histoire naturelle, Paris; MV, Museum of Victoria, Melbourne). **a)** *Microcardium aequiligratum*, length 26.5 mm, Philippines, MUSORSTOM-2 Stn 17 (MNHN); **b)** *Nemocardium bechei*, length 40.0 mm, New Caledonia Stn 542 (MNHN); **c)** *Pratulium thetidis*, length 16.0 mm, southern Australia (AMS C145776); **d)** *P. pulchellum*, length 22.0 mm, New Zealand (MNHN); **e)** *N. antisemigranulatum*, length 39.2 mm, middle Miocene of Australia (MV P128253-57); **f)** *N. edwardsi*, length 50.1 mm, Paleocene of Paris Basin (MNHN); **g)** *Frigidocardium* sp., length 10.9 mm, middle Miocene of Australia (MV P123418); **h)** *P. hemimeris*, length 8.6 mm, middle Miocene of Australia (MV P123419); **i)** *P. ornithopetronicum*, length 11.4 mm, upper Oligocene of Australia (MV P123422-36); **j)** *P. proterothetidis*, length 12.4 mm, Pliocene of Australia (MV P30788); **k)** *Microcardium* sp. A, length 11.1 mm, north-western Australia (AMS C145746); **l)** *Microcardium* sp. B, length 11.3 mm, New Caledonia, MUSORSTOM-6 Stn DW428 (MNHN); **m)** *Trifaricardium* sp., length 11.6 mm, northeastern Australia (AMS C145814).

Pacific heading, but did not record *Nemocardium* from that region. Therefore, a re-evaluation of the generic status of Australian species seemed necessary to decide if there is any correlation between the past and the present biogeography of Protocardiines.

TAXONOMY

Nemocardium s.s. is well known from Cenozoic strata. It is a typical Tethyan Indo-Pacific element, with maximum species diversity in the Paleogene of Europe, where about 30 nominal species are recorded (Keen, 1950; Tremlett, 1950; Popov, 1977). *Nemocardium* exhibits a characteristic, strongly discrepant, outer sculpture with well marked radial ribs on the posterior area; the remainder of the surface is quite smooth and reveals only numerous, fine and low subsurface radial riblets. Internally, the marginal crenulations appear much stronger on the posterior area, in accordance with the outer radial sculpture. On the posterior margin, crenulations correspond with radial ribs but, on the ventral and anterior margins, they correspond with interstices.

In the Cenozoic of New Zealand, *Nemocardium* is represented by *Varicardium* Marwick, 1944, known from the Upper Eocene to the Middle Miocene (Beu and Marwick, 1990). Species of *Varicardium* have been first referred to the Mesozoic genus *Protocardia* (Suter, 1914; Finlay, 1924) in view of the concentric folds of their anterior slope. However, as already pointed out by Marwick (1944) himself, it appears to be a case of secondary convergence and *Varicardium* more probably represents an offshoot of the *Nemocardium* line in New Zealand (Boreham, 1965; Keen, 1980).

In Australia, *Nemocardium* is known from the Miocene onwards, represented by the species *N. antisemigranulatum* (McCoy, 1877) (Fig. 2e). McCoy (op. cit.) described it as a species of "*Cardium* (*Protocardium*)"; an unjustifiable emendation for *Protocardia*. However, this latter taxon is confined to the Mesozoic, and Stewart (1930) has given good reasons for separating it from *Nemocardium* at the generic level. Since then, this species has often been classed in *Pratulium* (Darragh, 1970). However, it shows strong affinities with the Recent species *N. bechei*, and with typical *Nemocardium* species from the Paleogene of Europe (Fig. 2f), and fits well in that genus. This kind of confusion in the generic placement of species is rather common in the Protocardiinae. The first confusion between *Nemocardium* and *Pratulium* was due to Iredale (1927) himself, who differentiated the Australian specimens of *N. bechei* under the name "*Pratulium probatum*", i.e. only three years after he created the genus *Pratulium*; the true affinity of *P. probatum* has been shown by Wilson and Stevenson (1977). In addition, Australian Cenozoic Protocardiines are almost invariably referred to *Pratulium*, without any consideration to their true generic status. For example, it was found that collections of the

Museum of Victoria contain a Miocene species of the genus *Frigidocardium* Habe, 1951 (Fig. 2g) which had been confused with *P. hemimeris* (Tate, 1887). This seems to be the first fossil record of *Frigidocardium* in Australia. *Frigidocardium* is characterized by an homogeneous outer sculpture, whereas both *Pratulium* and *Nemocardium* exhibit two clearly differentiated areas.

Recent species of *Pratulium* have a distinctive sculptural pattern. As noted above, the outer surface has two clearly differentiated areas as in *Nemocardium* s.s. but, in *Pratulium*, radial ribbing is not confined to the posterior area. Moreover, the anterior and median areas of *Pratulium* spp. have a fine secondary sculpture of irregularly concentric, anastomosing threads crossing the radial ribs. The marginal crenulations are not clearly stronger posteriorly as they are in *Nemocardium* but, as in that genus, the crenulations do correspond with the radial sculpture. The study of Australian fossils reveals that, despite the above mentioned errors in generic placement, *Pratulium* actually is present in Australia during the whole Cenozoic, and probably also in Upper Cretaceous (Skwarko, 1983). The known Australian Cenozoic species referable to *Pratulium* are: *Cardium hemimeris* Tate, 1887 (Fig. 2h), *Protocardia ornithopetronica* Chapman and Crespin, 1928 (Fig. 2i), *N. (Pratulium) proterothetidis* Ludbrook, 1955 (Fig. 2j). In New Zealand, *Pratulium* also has a long history (Finlay and Marwick, 1937; Marwick, 1944) and there is evidence that it has been present from the Early Paleocene onwards (Hornibrook and Harrington, 1957; Beu and Marwick, 1990). However, none of the Cenozoic European species referred formerly to *Pratulium* actually belong to this genus (Gilbert and Van de Poël, 1970).

Thus, *Pratulium* appears to be a distinctive genus with a mainly Australian-New Zealand distribution, and the claim that it represents a Tethyan Indo-Pacific element seems unfounded. Recent *Pratulium* comprises three species, one in New Zealand and two in Australia (one undescribed).

In spite of morphological similarities between *Partulum* and *Microcardium* Thiele, 1934, the latter seems to be a distinct lineage of tropical affinities, with a completely different history. Its primary diversification center could be Central America. Morphologically, *Microcardium* can be distinguished from *Pratulium* by the occurrence of concentric scales in the interstices of radial ribs of the posterior area. *Microcardium* is here reported for the first time in the extant Australasian fauna, with one new species in Australia (Fig. 2k), and one in New Caledonia (Fig. 2l). There do not seem to be any fossil records of this genus in Australia. *Trifari-cardium* Kuroda and Habe, 1951, is also recorded for the first time in that region, with a new Recent species occurring in Australia and the Coral Sea (Fig. 2m). This genus closely resembles *Frigidocardium*, but can be distinguished by its strong, beaded concentric threads on the anterior slope.

The other Australian species of the subfamily Proto-

cardiinae can be referred to *Frigidocardium* (two species) and *Lyrocardium* (one species), with a single species (*Nemocardium bechei*) remaining in *Nemocardium s.s.* In their comprehensive account of Western Australian Cardiidae, Wilson and Stevenson (1977) dealt with these taxa, but described the species of *Frigidocardium* under the heading of '*Nemocardium (Microcardium)*'.

DISCUSSION

If the subfamily Protocardiinae represents a mainly deep-water, relict group in the Recent fauna, it appears to be well diversified in Australasia. The bathymetrical distribution of its member taxa reveals once more the conservative character of the upper bathyal zone (Lozouet, 1990). Moreover, the recent exploration of the bathyal area of South West Pacific around New Caledonia reveals that a number of taxa descend directly from the Mesozoic fauna (Vacelet, 1977; Ameziane-Cominardi *et al.*, 1987; Bouchet, 1987; Bourseau *et al.*, 1987) in this relatively stable, refuge area, which represents a survival of the eastern rim of Gondwanaland. In itself, this new information justifies the efforts of faunistic research made in this region.

ACKNOWLEDGMENTS

I wish to thank Philippe Bouchet (Muséum national d'Histoire naturelle, Paris), Thomas Darragh (Museum of Victoria, Melbourne), George Kendrick (Western Australian Museum, Perth), Neville Pledge (South Australian Museum, Adelaide), Winston Ponder (Australian Museum, Sydney) and Shirley Slack-Smith (Western Australian Museum, Perth) for communication of material on which this study is based. I am also grateful to Thomas Darragh for valuable assistance in Australian biostratigraphy and relevant literature.

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Date of manuscript acceptance: 31 October 1991

Preliminary cladistic analysis of the bivalve family Cardiidae

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Abstract. Phylogenetic relationships within the bivalve family Cardiidae have been examined by cladistic analysis. Thirty-six of the approximately 180 cardioid supraspecific taxa are analyzed, including members of each of the generally recognized cardiid subfamilies, plus the cardioid families Lahilliidae, Lymnocardidae, and Tridacnidae. Data for each taxon have been taken from a single species. For the outgroup, a hypothetical ancestor has been constructed from data for the carditids *Cyclocardia ventricosa* (Gould) and *Cardita variegata* Bruguière. The data consist of 54 characters and 170 character states. Results indicate that the families Lahilliidae, Lymnocardidae, and Tridacnidae should be given subfamilial status within the Cardiidae. *Septocardia* is removed from the Cardiinae and placed in its own subfamily, and the subfamily Protocardiinae is found to be paraphyletic. The Laevicardiinae, as proposed by Keen (1936, 1951, 1969, 1980), is shown to be polyphyletic: *Cerastoderma* is a lymnocardiine; *Dinocardium* is a cardiine; *Clinocardium* is the type genus of the Clinocardiinae. In addition to *Laevicardium*, only *Habecardium* and *Fulvia* remain in Laevicardiinae. The Trachycardiinae is found to be a monophyletic taxon within the Cardiinae. The subfamilies Clinocardiinae, Tridacninae, Lymnocardidae, and Fraginae form a monophyletic clade. *Sawkinsia*, long considered a tridacnid, belongs with the Cardiinae.

Bivalves of the family Cardiidae, or cockles, display a wide spectrum of shell shapes, ribbing and ornamentation patterns, hinge morphologies, and numerous other conchological features. Their complex morphology, accompanied by their good fossil record, allows the cardiids to be evaluated evolutionarily, ecologically, functionally, and phylogenetically in considerable detail.

The higher-level taxonomy of the Cardiidae has been more thoroughly studied than that of most other groups of bivalves (Dall, 1901; Stewart, 1930; Keen, 1936, 1937, 1951, 1969a, 1980; Fischer-Piette, 1977; Kafanov and Popov, 1977; Popov, 1977; Wilson and Stevenson, 1977; Voskuil and Onverwagt, 1989). Kafanov and Popov (1977) made the only detailed attempt to reconstruct the phylogenetic history of the group.

Traditionally, the superfamily Cardioidea comprises: (1) the Cardiidae; (2) the extinct, southern hemisphere Lahilliidae; (3) the brackish-water Lymnocardidae, confined to eastern Europe and southwestern Asia; (4) the Tridacnidae, or giant clams [Keen (1969b), Kafanov and Popov (1977), and Scarlato and Starobogatov (1979) have placed the giant clams in a separate superfamily]. Kafanov and Popov (1977) contended that the Lahilliidae belonged to the Arcticoidea. However, as noted by Finlay and Marwick (1937) and Marwick (1944), the hinge of *Lahillia* is of the cardiid, not arcticoide type; the lack of external ornament is apparently a case of convergence. This classification has not been taken for granted, and representatives of the Tridacnidae, Lahilliidae, and Lymnocardidae are included in this analysis.

Additionally, *Cardium acuticostatum* d'Orbigny, 1842, is included. Wilckens (1904) placed the Cretaceous *C.*

acuticostatum in *Cardium* (*Bucardium*), which Keen (1980) indicates is known from only the Miocene to Recent.

Boss (1971), Kafanov and Popov (1977), Keen (1980) and Ponder *et al.* (1981) placed the enigmatic *Hemidonax* in the Cardioidea. However, Scarlato and Starobogatov (1979) argued that *Hemidonax* is aligned with the Donacidae. After examination and comparison of the shell and of the anatomy (both external and internal) of *Hemidonax* to both cardiids and donacids, I cannot justify placing *Hemidonax* as a member of the Cardioidea. However, neither can I place *Hemidonax* within the Donacidae. Instead, I favor placing *Hemidonax* as *incertae cedis* within the order Veneroida, until a phylogenetic analysis of the Veneroida is undertaken.

Virtually all cardiid taxonomy is based on hard parts, with the exception of Starobogatov's (In: Kafanov and Popov, 1977) study of stomach structure. Most of what is known about the anatomy of cardiids comes from the study of the common cockle, *Cerastoderma edule* (Linnaeus) (see Ménégau, 1890; Johnstone, 1899; Zugmayer, 1904; Küpfer, 1915; Roche, 1925; Atkins, 1937; Graham, 1949), which is taken as a model for the entire family. Furthermore, Russian malacologists (Kafanov and Popov, 1977; Popov, 1977; Taktakishvili, 1987) place *Cerastoderma* within the subfamily Lymnocardidae - which, less *Cerastoderma*, Keen (1969a, 1980) considered to be a separate family within the superfamily Cardioidea. The only treatment of comparative anatomy of the cardiids is that of Pelseneer (1911).

The goals of this study are to: (1) determine the status and content of subfamilies erected by previous workers; (2) propose a preliminary phylogenetic hypothesis for the family.

Characters and character states are briefly described herein and will be treated fully in future publications.

MATERIALS AND METHODS

There are about 180 generally accepted cardioid genera and subgenera (see Keen, 1969a, 1980; Kafanov and Popov, 1977; Vokes, 1980; Taktakishvili, 1987). It is not feasible currently to run a computer-driven cladistic program for such a large number of taxa. The 36 taxa chosen in this study include at least one representative of each of the cardioid subfamilies accepted by Keen (1969a, 1980), Kafanov and Popov (1977), and Voskuil and Onverwagt (1989). As stated above, the tridacnids (*Tridacna*), lahilliids (*Lahillia*), and lymnocardiids (*Hypanis*) are also represented in this analysis (the suffix -ids is used in a vernacular sense until their taxonomic placement is discussed thoroughly). Kafanov and Popov's (1977) and Keen's (1980) classification scheme for the taxa analyzed in the present study is given in appendices 1 and 2.

A cladistic analysis of the 36 taxa with 54 characters comprising 170 character states (appendices 3 and 4) was made using PAUP 3.0d (Swofford, 1989). The accelerated transformation option (ACCTRAN) was used, and steps were not added to terminal taxa with polymorphisms. Synapomorphies for each node are presented in appendix 5.

Character states were encoded from a single species of each genus or subgenus. Most of the taxa are represented by their type species. Exceptions are those taxa for which

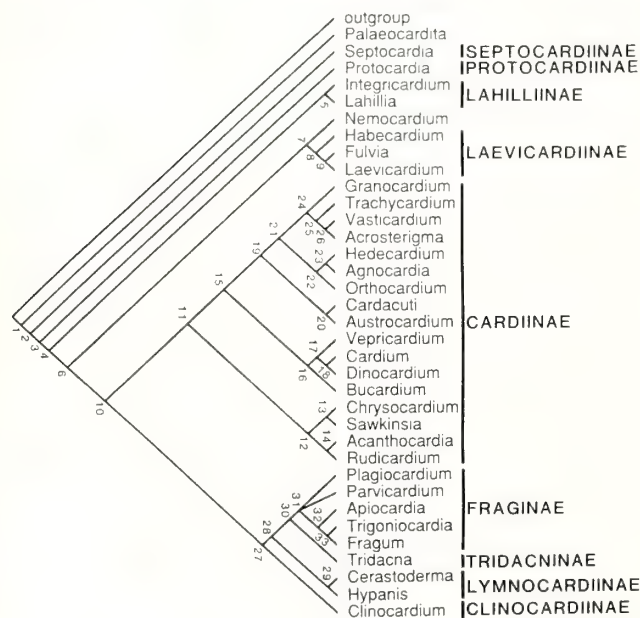


Fig. 1. Majority-rule consensus tree of 50 most parsimonious trees. Nodes 21 and 22 supported by 60% of trees; nodes 28 and 30 supported by 70% of trees; node 31 supported by 90% of trees; all other nodes supported by 100% of trees. Synapomorphies supporting each node given in appendix 5.

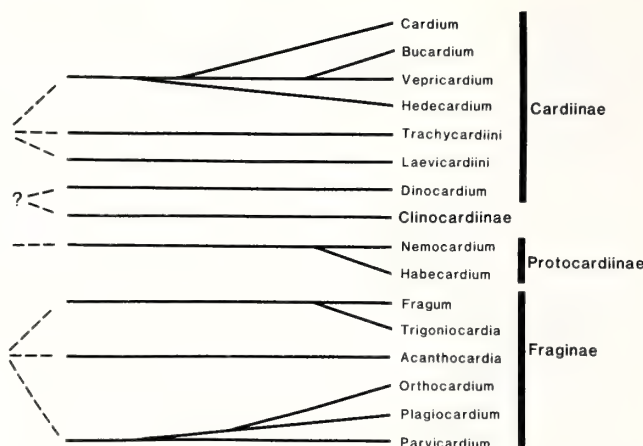


Fig. 2. Evolutionary scenario for the Cardiidae from Kafanov and Popov (1977). Only those taxa included in both the present study and Kafanov and Popov (1977) are shown.

(1) material of the type species was unavailable, or (2) anatomical material was not available for the type (e.g. *Nemocardium*, for which the type species is extinct), but was available for another species generally assigned to that taxon. Taxa represented by species other than the type are listed in appendix 6. Therefore, this analysis should be taken as a phylogeny for these species only. Character states presented may not be constant throughout all species of a given genus. Because there is considerable disagreement over what constitutes a genus or a subgenus in the Cardiidae, all terminal taxa will be considered to have equal rank as genera; no distinction will be made between genera and subgenera, except as noted in the text.

Citations in the character list (appendix 3) refer to previous discussions of that character. Except for the information on *Cyclocardia* (see below), character 12, and character 8 for *Parvicardium* (from Pelseneer, 1911), all character states were encoded from examination of specimens.

The Cardiidae are generally accepted as having been derived from a member of the Carditoidea [Cox, 1949; Keen, 1969a, 1980; Newton, 1986; but see Morris (1978) and Morris *et al.* (1991)]. These authors have postulated an evolutionary scenario of *Palaeocardita* originating from some primitive carditid or permophorid, with *Septocardia* then originating from *Palaeocardita*. *Palaeocardita* is usually placed with the Carditidae (Chavan, 1969). However, examination of the one species of *Palaeocardita* available to me, *Palaeocardita silberlingi* Newton *et al.*, has led me to place this species within the ingroup Cardiidae, on the basis of its cardinal teeth, which are arranged as in *Septocardia* and *Protocardia* [see Newton *et al.* (1987) for a discussion of this species]. Therefore, to represent the outgroup, a hypothetical ancestor was constructed with information from the Recent carditids *Cardita variegata* Bruguière and *Cyclocardia ventricosa*

(Gould). Character states for *C. ventricosa* were taken from information in Yonge (1969). For characters 6 and 30, the two carditids provided conflicting information, and hence the states are scored as missing ("?").

Most characters are unordered. It was possible to construct character state trees based on ontogeny for characters 23 (shell shape), 24 (ribbing pattern), 29 (mosaicostacum), and 40 (rib flares).

RESULTS AND DISCUSSION

Fifty most parsimonious trees of 208 steps (consistency index = 0.566) were found. The 50% majority-rule consensus tree is presented in figure 1, which can be compared with two previously presented evolutionary scenarios. Kafanov and Popov (1977) produced a phylogram based on two key characters: stomach structure (analyzed by Ya. I. Starobogatov) and Popov's (1977) work on shell microstructure. Kafanov and Popov (1977) considered 38 taxa [only those taxa represented in both my analysis and that of Kafanov and Popov (1977) are shown in figure 2; they considered neither the origin of the Cardiidae nor the group's Mesozoic history]. Only nine of the 28 extant taxa were examined for stomach structure. Starobogatov's study of stomach structure rests heavily on the presence/absence and position of sorting areas, as described by Purchon (1960a). Purchon's (1960a) description of the cardiid stomach came from the study of *Cerastoderma edule* by Graham (1949). Starobogatov (In: Kafanov and Popov, 1977) stated that the SA-3, or posterior sorting area, is absent in *Cerastoderma* based on examination of *C. glaucum* (Bruguère) (Ya. I. Starobogatov, pers. comm.) and *Hypanis*. However, the posterior sorting area (labeled SAP) is the most prominent structure in Graham's (1949) figure of the stomach of *C. edule*. While promoting the utility of using stomach structure to elucidate the higher-level phylogeny of the Bivalvia (Purchon, 1959, 1960a, b), Purchon (1960a:481) warns that "...it is not easy to make an objective analysis of the occurrence and identities of the various sorting areas. The presence or absence, and the degree of development of the various sorting areas has a profound effect on the appearance of the interior of the stomach, and could obscure more fundamental issues such as the course taken by the major typhlosole and the intestinal groove...the occurrence of sorting areas can only be used with the greatest caution for phylogenetic purposes." Although seven of the 20 anatomical characters in the present analysis concern the stomach, none relate to the sorting areas.

Popov's (1977, 1986) classification of bivalve shell microstructure conflicts with those of Carter (1980, 1989), Carter and Clark (1985), Carter and Lutz (1989) and Watabe (1984). The only systemically useful microstructural characters that I have found so far concern the relationship of the ornament to the rest of the shell (characters 28, 29 and 40).

The only cardiid phylogeny suggested by Keen is found in her description of cardiid evolution (Keen, 1980). I have constructed a phylogram (Fig. 3) based on that description.

In the present analysis, *Palaeocardita silberlingi* is located at the base of the cladogram, followed by *Septocardia*, *Protocardia*, and then the rest of the Cardiidae. This topology is in agreement with the ideas of early cardiid evolution suggested by Cox (1949), Keen (1969a, 1980) and Newton (1986). However, the monophyly of *Palaeocardita* is questionable (C. R. Newton, pers. comm.), and the more common species, including the type *P. austriaca* (Hauer) and *P. crenata* (Goldfuss) could be carditoids, whilst *P. silberlingi* is a cardiid. Due to the uncertainty of the taxonomy of *Palaeocardita*, I refrain from placing *P. silberlingi* in a higher taxon within the Cardiidae.

Septocardia was placed in its own family, the Septocardiidae, in the superfamily Tridacnoidea by Kafanov and Starobogatov (In: Kafanov and Popov, 1977). *Septocardia* is clearly a primitive cardiid and it does not share any of the derived features of *Tridacna*. Likewise, *Septocardia* does not belong in the derived subfamily Cardiinae as indicated by Keen (1969a, 1980). *Septocardia* is here placed in the cardiid subfamily Septocardiinae.

The subfamily Protocardiinae has been understood to include the genera *Protocardia*, *Integricardium*, *Jurassicardium*, and *Nemocardium* (Kees, 1969a, 1980). My results indicate that this is a paraphyletic group. *Integricardium* is more closely related to *Lahillia*. The paraphyly of the Protocardiinae has been acknowledged implicitly for some time. McLearn (1933) erected *Onestia* as a subgenus of *Integricardium*; the former was considered a genus by McLearn

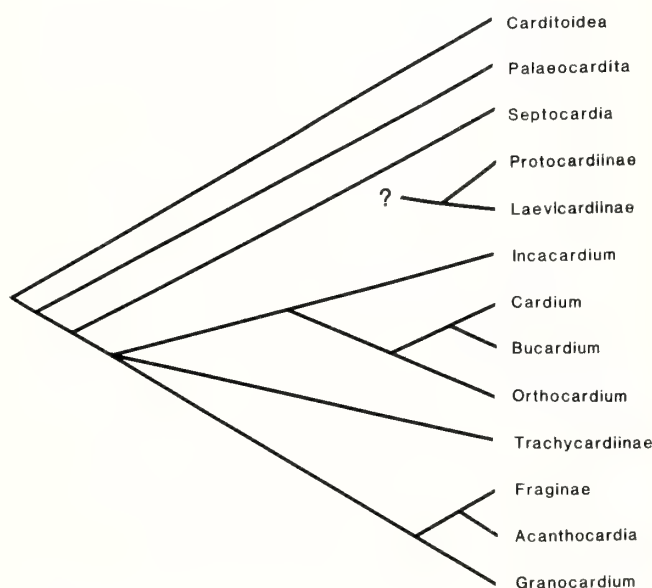


Fig. 3. Evolutionary scenario reconstructed from Keen (1980:24-30).

(1945) and Day (1978), but not by Keen (1969a, 1980). Day (1978) postulated that *Integricardium* is ancestral to *Onestia*, which is in turn ancestral to *Lahillia*. He also placed *Onestia* in the Lahilliidae. Present results indicate that the family Lahilliidae should be relegated to a subfamily (Lahilliinae) within the family Cardiidae (as originally proposed by Finlay and Marwick, 1937), and should include *Integricardium*.

Nemocardium is the sister taxon to the Laevicardiinae. This group is in turn the sister taxon to the rest of the cardiids. Because of its change in ribbing pattern from that similar to *Nemocardium* as a juvenile, to that of *Fulvia* as an adult, *Habecardium* has been recognized as transitional from the Protocardiinae to the Laevicardiinae (Glibert and van de Poel, 1970; Keen, 1980). Glibert and van de Poel (1970) erected *Habecardium* as a subgenus of *Laevicardium*, into which some of the species of *Habecardium* had been placed previously. Keen (1980) placed *Habecardium* as a subgenus of *Nemocardium*. Popov (1977) and Kafanov and Popov (1977) also placed *Habecardium* as a subgenus of *Nemocardium*, but did not recognize it as transitional to *Laevicardium* and *Fulvia*, placing the latter two taxa in the Cardiinae. Besides ribbing pattern (24:2), the Laevicardiinae are united by the number of ctenidial plicae (5:2), tentacles that extend only to the bottom of the posterior adductors (10:0), presence of complex eyes (12:1), a centrally located right caecum (18:1), and shape of the cardinal teeth (43:3 and 45:3).

Keen (1969a, 1980) placed all the Cenozoic proto-cardiines in the genus *Nemocardium*. Other authors (Fischer-Piette, 1977; Popov, 1977; Wilson and Stevenson, 1977; Noda, 1988; Voskuil and Onverwagt, 1989) have raised some of the subgenera to the generic level. It is suspected strongly that the subtraction of *Habecardium* from *Nemocardium* would leave the latter as a monophyletic group. I decline here to place *Nemocardium* within a subfamily. A systematic analysis which includes all of the subgenera of *Nemocardium* as in Keen (1969a, 1980) represented, plus the Laevicardiinae, is in progress.

Jurassicardium is a monotypic taxon known from only a few specimens. Only the type material is sufficiently well preserved to be of systematic use, and I have not examined it.

The remainder of the Cardiidae comprise those forms typically accepted to constitute the taxa Cardiinae, Trachycardiinae, Fraginae, Clinocardiinae, Lymnocardiidae, and Tridacnidae. Two monophyletic clades can be distinguished within this unnamed taxon. One clade, here considered the subfamily Cardiinae, contains the taxa placed in the Trachycardiinae and most of the taxa placed in the Cardiinae by Keen (1969a, 1980), and all of taxa placed in the Cardiinae (except for *Laevicardium* and *Fulvia*) by Kafanov and Popov (1977). In agreement with Kafanov and Popov (1977), the present results indicate that *Trachycardium* and the related taxa *Acrosterigma* and *Vasaticardium* do not constitute a separate subfamily but are members of the Cardiinae.

The least derived monophyletic group within the Cardiinae contains the taxa *Acanthocardia*, *Rudicardium*, *Sawkinsia*, and *Chrysocardium*. Synapomorphies of this clade are cardiiform shell shape (23:5), tuberculate spines (28:5) and irregular cross-striae (30:1). *Rudicardium* is considered either a subgenus of *Acanthocardia* (Keen, 1969, 1980; Popov, 1977) or a synonym of it (Voskuil and Onverwagt, 1989). These two taxa are united by a suite of hinge characters: incomplete anterior cardinal socket (42:1); shape of the cardinal teeth (43:8 and 45:10); hinge plate overlapping the right posterior lateral socket (48:1).

Cox (1941) erected *Sawkinsia* as a genus of cardiid. Vokes (1953) placed *Sawkinsia* in the Tridacnidae, and was followed by Rosewater (1965), Keen (1969b), and Jung (1976). Stasek (1962) considered the resemblance between *Sawkinsia* and the tridacnid *Hippopus* to be a case of convergence. *Sawkinsia* does not share any of the derived characters of *Tridacna*, nor any of *Tridacna*'s notable autapomorphies: (1) there is no loss of the anterior lateral teeth; (2) the spines are tubercles, not wide and gently curved; (3) nor is there any evidence of the rotation of the shell about the animal. According to the present phylogenetic hypothesis, *Sawkinsia* is a member of the subfamily Cardiinae.

Woodring (1982) erected the genus *Chrysocardium* in the subfamily Fraginae based on a single left valve. *Chrysocardium* shares not only a lunule flap touching the beak (25:3) with *Sawkinsia*, but three characters found nowhere else in the Cardiidae: hinge inversion (36:1) (described for *Sawkinsia* by Cox, 1941); weak myophorous buttress (38:1); double keel (41:1). *Chrysocardium* should be considered a synonym of *Sawkinsia*, however *C. aurum* Woodring, appears to be valid. Except for missing data, the characters for two taxa are scored identically (see appendix 4).

The next monophyletic group includes *Bucardium*, *Cardium*, *Vepricardium*, and *Dinocardium*, and is united by seven synapomorphies. The close relationship of the first three taxa to each other has been recognized by numerous authors (see Keen, 1969a; Kafanov and Popov, 1977). The position of *Dinocardium*, however, remains uncertain. Keen (1951, 1969a, 1980) placed *Dinocardium* in the Laevicardiinae. Kafanov and Popov (1977), in dismantling the Laevicardiinae, tentatively placed *Dinocardium* in the Cardiinae; Kafanov (1980:298) called the taxonomic position of *Dinocardium* "most mysterious."

The next node within the Cardiinae contains *Austrocardium* and *Cardium acuticostatum*, and is united by lack of lunule flap (25:0) and shape of the anterior cardinal (45:5). Freneix and Grant-Mackie (1978) erected the Cretaceous *Austrocardium* as a monotypic taxon. Wilckens (1904) placed the Cretaceous form *C. acuticostatum* in *Cardium* (*Ringicardium*) [= *Cardium* (*Bucardium*)], which is otherwise a Miocene to Recent taxon (Keen, 1969a, 1980). The results of my analysis indicate that *C. acuticostatum* belongs in

Austrocardium. There are three other Cretaceous species that differ little from *C. acuticostatum* and *Austrocardium*. These are: (1) *C. denticulatum* Baily, which was placed by Dartevelle and Freneix (1957) in *Acanthocardia* (*Acanthocardia*); (2) *C. (Bucardium) lillei* Freneix and Grant-Mackie (specimens of which had originally been described as *C. acuticostatum*); (3) *Schedocardia ? waiparana* Freneix and Grant-Mackie. These species should be placed provisionally in *Austrocardium*, as they share the apomorphies of *Austrocardium* but not those of either *Bucardium* or *Acanthocardia*. Except for cases of missing data, the posterior gape of *C. acuticostatum* (33:1) is the only character not scored identically to that of *Austrocardium* (appendix 4). The posterior gape is convergent with that of *Cardium*.

Hedecardium, *Orthocardium*, and *Agnocardia* form a monophyletic clade. *Hedecardium* has been considered variously as a subgenus of *Vepricardium* (Keen, 1969a, 1980), a genus closely related to *Vepricardium* (Popov, 1977), and a genus in the Protocardiinae (Marwick, 1960; Maxwell, 1978). The latter authors derived *Hedecardium* from *Nemocardium* on the basis of its discrepancy in thickness in the ribs across the shell. However, the rib discordance in *Hedecardium* is not comparable to that in *Nemocardium*. In *Hedecardium*, four to six ribs on the posterior slope are split with a furrow running down the middle, and the remaining posterior ribs are reduced in strength (as happens numerous times in the Cardiidae; it was the basis of Keen's [1936] subfamily Laevicardiinae, shown to be polyphyletic). This change from all ribs of equal width to the rib discrepancy seen in *Hedecardium* can be seen in the growth stages of a single shell. Likewise, the early growth stages of *Hedecardium* are circular, and circular shells are unknown in any form of *Nemocardium*. As a juvenile, *Hedecardium* would strongly resemble *Orthocardium*. It is recommended that *Hedecardium* and *Orthocardium* be considered as distinct genera.

Orthocardium has been considered a subgenus of *Vepricardium* (Keen, 1969a) or of *Cardium* (Keen, 1980) or a genus of fragine (Popov, 1977; Kafanov and Popov, 1977). Here, *Orthocardium* is united with *Agnocardia* and *Hedecardium* by concave ribs (32:1), a condition otherwise unknown in the Cardiidae.

The last clade within the Cardiinae comprises *Grano-cardium*, *Trachycardium*, *Acrosterigma*, and *Vasticardium*, and is united by one character, ovate shell shape (23:3). The latter three taxa, united by three synapomorphies, are usually placed in the Trachycardiinae (Keen, 1969a, 1980) or the tribe Trachycardiini (Kafanov and Popov, 1977) within the Cardiinae. Current results support the latter.

The other major clade of cardiids is united by five synapomorphies and generally contains forms that have been assigned to the Clinocardiinae, Lymnocardiidae, Tridacnidae, and Fraginae. Of these the least derived is *Clinocardium*. This taxon was placed in the Laevicardiinae by Keen (1951, 1969a,

1980), but has come to be accepted as the type of the subfamily Clinocardiinae (Kafanov and Popov, 1977; Kafanov, 1980; Voskuil and Onverwagt, 1989). The next node encountered is *Cerastoderma* and *Hypanis*, which is the sister taxon to the *Tridacna* and Fraginae. Synapomorphies of lymnocardiids + *Tridacna* + Fraginae are medium labial palps (1:1), functional byssus in adult (6:2), posterior cardinal socket angle (44:1) and shape of the anterior cardinal (45:9). Yonge's (1936) and Stasek's (1962) suggestion that the ancestry of *Tridacna*'s was close to that of *Cerastoderma* is upheld by the results. Giant clams should be considered as the subfamily Tridacninae within the Cardiidae.

Cerastoderma and *Hypanis* form a monophyletic group. Therefore, as has been argued by eastern European malacologists for some time (Kafanov and Popov, 1977; Popov, 1977; Taktakishvili, 1987; Basch, 1990), the brackish-water forms should be subfamily Lymnocardiinae, and contain *Cerastoderma*. Furthermore, the results support Kafanov and Popov's (1977) contention that the Lymnocardiinae are related closely to the Fraginae.

Five of the seven characters that unite *Tridacna* and the Fraginae are anatomical: tentacle pattern (9:2); large valvule (11:2); centrally located style sac (15:1); presence of a raised bar on the stomach floor (17:1); posteriorly located left caecum (19:0). The Fraginae is united by the presence of ventral appendages on the foot (7:1), absence of a periphonal suture (8:1), and presence of a mosaicostracum (29:1). The least derived taxa, *Plagiocardium* and *Parvicardium*, were placed in the Fraginae by Kafanov and Popov (1977) but in the Cardiinae by Keen (1969a, 1980). *Apiocardia*, *Trigoniocardia*, and *Fragum* are united by eight synapomorphies, all based on hinge characters. Finally, *Trigoniocardia* and *Fragum* are united by six synapomorphies, five of which are anatomical characters: short labial palps (1:0); fewer than ten ridges on the palps (2:1); the inner palp connected to the bottom of the inner demibranch (3:1); fewer than 20 ctenidial plicae (5:0); type 4 gut (13:3); quadrate shell shape (23:2).

From the above discussion it can be concluded that the family Cardiidae includes nine subfamilies: Septocardiinae; Protocardiinae; Lahilliinae; Laevicardiinae; Cardiinae; Clinocardiinae; Tridacninae; Lymnocardiinae; Fraginae. Taxa usually assigned to the Trachycardiinae form a monophyletic group within the Cardiinae. *Dinocardium* is a cardiine closely related to *Cardium* and *Vepricardium*. *Sawkinsia* (= *Chrysocardium*) is transferred from the Tridacninae to the Cardiinae, as it is related closely to *Acanthocardia* and *Rudicardium*.

The Protocardiinae (as presented in the Treatise on Invertebrate Paleontology) is paraphyletic. *Integricardium* is a member of the Lahilliinae. *Nemocardium* is the sister taxon to the Laevicardiinae. The subfamilies Clinocardiinae, Tridacninae, Lymnocardiinae, and Fraginae form a monophyletic group.

ACKNOWLEDGMENTS

Numerous people and institutions have allowed me to examine their collections and have lent specimens. Foremost, I would like to thank the Academy of Natural Sciences of Philadelphia, my supervisor at the Academy, G. Rosenberg, and G. Davis and the rest of the staff of the departments of Malacology and Paleontology of that institution. I would like to thank the following people and institutions for allowing me to examine their collections: T. Waller, W. Blow, R. Hershler, R. Houbbrick, J. Harasewych (United States National Museum); M. Hinckley, N. Eldredge (American Museum of Natural History); C. Coney (Los Angeles County Museum of Natural History); M. Baker, M. Nitecki, J. Voight, R. Bieler (Field Museum of Natural History). I would like to thank the following people for lending specimens: W. Ponder, Australian National Museum; A. Beu, New Zealand Geological Survey; J. A. Grant-Mackie, University of Auckland; S. Freneix, J. -C. Fisher, and Y. Gayraud, Museum National d'Histoire Naturelle, Paris. I examined specimens of *Palaeocardita* and *Septocardia* at C. R. Newton's laboratory at Syracuse University. I learned techniques for the study of shell microstructure at J. G. Carter's laboratory at the University of North Carolina-Chapel Hill.

The following agencies have generously given me financial assistance for my research: The Academy of Natural Sciences of Philadelphia (Jessup Fund), the National Capitol Shell Club, the Western Society of Malacologists, the Paleontological Society, Conchologists of America, the Santa Barbara Shell Club, the Hinds Fund of the University of Chicago, Sigma Xi, the Gurley Fund of the Department of Geophysical Sciences of the University of Chicago, and the Amoco Corporation. During part of my graduate study I was supported by a grant from the National Science Foundation (EAR-90-05744) to D. Jablonski. I would also like to thank the following people at the University of Chicago who have assisted me in my research: D. Jablonski, M. LaBarbera, O. Draughn, K. Roy, P. Wagner, and D. Miller.

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Date of manuscript acceptance: 25 September 1991

APPENDIX 1.

Classification of taxa used in this study following Kafanov and Popov (1977).
Taxa not listed are not discussed therein.

Superfamily Cardioidea

Family Cardiidae

Subfamily Protocardiinae

*Protocardium**Integricardium**Nemocardium**Habecardium*

Subfamily Cardiinae

Tribe Cardiini

Cardium

Tribe Vepricardiini

*Vepricardium**Agnocardia**Bucardium**Hedecardium*

Tribe Trachycardiini

*Trachycardium**Acrosterigma**Vasticardium*

Tribe Laevicardiini

Fulvia

Tribe Dinocardiini

Dinocardium

Subfamily Clinocardiinae

Tribe Clinocardiini

Clinocardium

Subfamily Fraginae

Tribe Fragini

*Trigoniocardia**Apiocardia**Fragum*

Tribe Acanthocardiini

*Rudicardium**Acanthocardia*

Tribe Parvicardiini

*Plagiocardium**Parvicardium**Orthocardium*

Subfamily Lymnocyrtinae

Tribe Cerastodermatini

Cerastoderma

Tribe Hypanini

Hypanis

Superfamily Tridacnoidea

Family Septocardiinae

Septocardia

Family Tridacnidae

Tridacna

Superfamily Arcticoidea

Family Lahillidae

Lahillia

APPENDIX 2.

Classification of taxa used in this study according to Keen (1969a, b; 1980). Taxa not listed are not considered in any of Keen's (1969a, 1969b, 1980) papers.

Superfamily Cardiacea

Family Cardiidae

Subfamily Cardiinae

*Septocardia**Granocardium**Vepricardium* (*Vepricardium*)*Vepricardium* (*Orthocardium*)*Vepricardium* (*Hedecardium*)*Cardium* (*Cardium*)*Cardium* (*Bucardium*)*Acanthocardia* (*Acanthocardia*)*Acanthocardia* (*Rudicardium*)*Acanthocardia* (*Agnocardia*)*Plagiocardium**Parvicardium*

Subfamily Trachycardiinae

*Trachycardium**Acrosterigma* (*Acrosterigma*)*Acrosterigma* (*Vasticardium*)

Subfamily Protocardiinae

*Protocardia**Integricardium**Nemocardium* (*Nemocardium*)*Nemocardium* (*Habecardium*)

Subfamily Fraginae

*Fragum**Trigoniocardia* (*Trigoniocardia*)*Trigoniocardia* (*Apiocardia*)

Subfamily Laevicardiinae

Laevicardium (*Fulvia*)*Laevicardium* (*Dinocardium*)*Cerastoderma**Clinocardium*

Family Lahillidae

Lahillia

Family Lymnocyrtidae

Hypanis

Superfamily Tridacnacea

Family Tridacnidae

*Tridacna**Sawkinsia*

APPENDIX 3.

List of characters and character states.

I. Anatomy

A. Labial palps

1. Length: 0) short, 1) medium, 2) long
2. Number of ridges on palps: 0) ridges absent, 1) <10, 2) 10 - 19, 3) 20 - 29, 4) >29
3. Connection of inner palp with ctenidia: 0) connects behind inner demibranch, 1) connects with bottom of inner demibranch

B. Ctenidia

4. Inner demibranch/outer demibranch relation: 0) Outer demibranch does not overlay inner demibranch, 1) Outer demibranch partially underlain by inner demibranch
5. Number of plicae: 0) <20, 1) 20 - 39, 2) 40 - 59, 3) 60 - 79, 4) 80 - 99, 5) >99

C. Foot

- Byssal apparatus: 0) absent in adult, 1) present in adult, but non-functional, 2) functional in adult (see Pelseneer, 1911)
7. Ventral appendages: 0) absent, 1) present

D. Siphons and tentacles

8. Periphonal suture: 0) present, 1) absent (see Pelseneer, 1911)
9. Tentacle pattern: 0) absent, 1) numerous, in both mantle fold and on siphonal area, 2) numerous, in mantle fold only, 3) few, in mantle fold and siphonal area, 4) few on siphonal area only
10. Dorsalmost extension of tentacles: 0) bottom of adductors, 1) to middle of adductors, 2) to top of adductors, 3) beyond top of adductors
11. Valvule (see Pelseneer, 1911) 0) absent, 1) small, 2) large
12. Eyes (see Kishinouye, 1894; Nagel, 1897; Zugmayer, 1904; Weber, 1908; Pelseneer, 1911; Braun, 1954) 0) simple, 1) complex

E. Gut

13. 0) Type 1, 1) Type 2, 2) Type 3, 3) Type 4, 4) Type 5

F. Stomach (see Graham, 1949 and Purchon, 1960a, for general descriptions of bivalve stomachs)

14. T3 (tertiary typhlosole): 0) absent, 1) present
15. Position of style sac: 0) posterior, 1) central
16. T1 (major typhlosole) curved (see Nakazima, 1964): 0) yes, 1) no
17. raised bar: 0) absent, 1) present
18. Position of right caecum: 0) right side of stomach, 1) central
19. Position of left caecum: 0) posterior to right caecum 1) caeca parallel, 2) anterior to right caecum
20. Accessory left caeca: 0) absent, 1) present

II. Shell

A. General

21. Posterior margin: 0) digitate, 1) crenulate, 2) smooth
22. Rib number: 0) absent, 1) less than 70, 2) greater than 70
23. shell shape: 0) carditaform, 1) quadrate - long, 2) quadrate - short, 3) ovate, 4) circular, 5) cardiiform, 6) oval, 7) cerastiform, 8) trigonal, 9) elliptical, 10) oblique

Character state tree: (((((5,(7,8)6)4,10,3)2,9)1)0

24. Anterior/central rib pattern: 0) concentric, 1) radial, equal in width to posterior ribs, 2) concentric, changing to radial, 3) rib discordance, 4) radial, thinner than posterior ribs, 5) none

Character state tree: (((2)4,5)0,3)1

25. Lunule flap (see Kafanov, 1980, pp. 298-299): 0) absent, 1) raised, does not block beak, 2) blocks beak but does not touch it, 3) touches beak, 4) strongly folded over beak
26. Ridges on lunule flap: 0) absent, 1) present
27. Growth line strength: 0) strong, 1) weak
28. Spines: 0) lumpy nodes, 1) none, 2) round knobs, 3) A-shaped, separate shell layer from ribs, 4) hollow posterodorsally, 5) tubercles, 6) gently curved, 7) hollow keel

Spines are defined as emanating from the top of the ribs. The "spines" of trachycardiines emanate from the side of the ribs, and are considered separately (character 40).

29. Mosaicostracum ("spines" on fragines): 0) none, 1) beads, 2) scales

Mosaicostracum was first described by Hamilton (1969). Keen (1980) and Vokes (1977, 1989) referred to this layer as the intritacalx (D'Attilio and Radwin, 1971). Carter (1989) considers intritacalx synonymous with mosaicostracum. This character is linearly ordered.

30. Cross-striae: 0) simple, 1) irregular, 2) absent
31. Internal rib expression: 0) weak, 1) strong
32. Ribs concave: 0) no, 1) yes
33. Posterior gape: 0) absent, 1) present
34. Dorsal nymph extension, 0) absent, 1) present
35. Posterior umbonal buttress: 0) absent, 1) present
36. Hinge inversion: 0) absent, 1) present
37. Nymph groove: 0) absent, 1) present
38. Myophorous buttress: 0) strong, 1) weak, 2) absent
39. Adductor scar relief: 0) strong, 1) weak
40. Rib flares ("spines" on trachycardiines): 0) absent, 1) strong, 2) weak

This character is linearly ordered.

41. Double keel: 0) absent, 1) present

B. Hinge teeth

- 1) Right cardinal teeth
42. Anterior socket: 0) complete, 1) incomplete
43. Posterior cardinal shape: 1) 1, 2) 2, 3) 3, 4) 4, 5) 5, 6) 6, 7) 7, 8) 8, 9) 9
44. Angle of posterior cardinal socket to horizontal: 0) greater than 40 degrees, 1) equal or less than 40 degrees
45. Shape of anterior cardinal: 0) 0, 1) 1, 2) 2, 3) 3, 4) 4, 5) 5, 6) 6, 7) 7, 8) 8, 9) 9

Rest of hinge

46. Right anterior lateral teeth - ventral tooth continues up into umbo: 0) no, 1) yes
47. Right anterior lateral teeth - ventral tooth inserts into socket: 0) no, 1) yes
48. Right posterior laterals: hinge plate overlaps socket: 0) no, 1) yes
49. Anterior lateral teeth: 0) absent, 1) present
50. Left posterior lateral teeth: 0) weak or absent, 1) strong
51. Nymph overlies posterior cardinals: 0) no, 1) yes
52. Left anterior lateral horizontal: 0) no, 1) yes
53. Left anterior lateral socket: 0) absent or weak, 1) strong
54. Left posterior lateral socket: 0) absent or weak, 1) strong

Data matrix for cladistic analysis. "?" signifies missing data. X indicates character state 10. A-D indicate polymorphisms. A: states 1 and 2; B: states 0 and 1; C: states 2 and 3; D: states 1, 2, and 3.

[illegible]

APPENDIX 5.

Synapomorphies for interior nodes. Nodes numbered as in figure 1. Terminal taxa not diagnosed.

Node	Synapomorphies (Character:State)
1	2:2, 5:1, 9:1, 11:1, 12:1, 14:0, 16:1, 20:0, 43:1
2	23:1, 25:1
3	24:0, 28:1, 30:2, 38:2, 49:1
4	27:1
5	21:2, 22:0, 23:9
6	23:2, 43:2, 45:2
7	24:4, 26:3, 48:1
8	5:2, 10:0, 12:1, 18:1, 24:2, 43:3, 45:3
9	39:1, 42:1
10	1:2, 6:1, 13:2, 19:1, 23:4, 24:1
11	5:4, 10:3, 25:2
12	23:5, 28:5, 30:1
13	25:3, 36:1, 38:1, 41:1
14	42:1, 43:8, 45:10, 48:1
15	21:0
16	18:1, 25:4, 26:1, 31:1, 34:1, 39:1, 42:2, 48:1
17	37:1, 46:1
18	2:3, 5:5, 23:5
19	4:0, 19:2, 50:1
20	25:0, 45:5
21	28:2, 30:0
22	32:1
23	42:1
24	23:3
25	25:1, 28:1, 40:1
26	10:2, 40:2, 43:4, 45:6
27	9:3, 23:6, 42:1, 43:5, 45:4
28	1:1, 6:2, 44:1, 45:9
29	13:4, 23:7
30	9:2, 11:2, 15:1, 17:1, 19:0, 23:8, 30:0
31	7:1, 8:1, 29:1
32	42:0, 43:6, 47:1, 50:2, 51:1, 52:1, 53:1, 54:1
33	1:0, 2:1, 3:1, 5:0, 13:3, 23:2

APPENDIX 6.

Taxa represented by species other than type.

- Palaeocardita*, type species *Palaeocardita austriaca* (Hauer). Species examined: *Palaeocardita silberlingi* Newton *et al.*
- Integricardium*, type species *Integricardium dupinianum* (d'Orbigny). Species examined: *Integricardium globulum* (Whitfield).
- Lahillia*, type species: *Lahillia angulata* (Philippi). Species examined: *Lahillia larseni* (Sharman and Newton).
- Nemocardium*, type species *Nemocardium semiasperum* (Deshayes). Species examined: *Nemocardium beckeii* (Reeve).
- Laevicardium*, type species *Laevicardium oblongum* (Gmelin). Species examined: *Laevicardium laevigatum* (Linne).
- Granocardium*, type species *Granocardium carolinum* (d'Orbigny). Species examined: *Granocardium dumosum* (Conrad).
- Agnocardia*, type species *Agnocardia claibornense* (Aldrich). Species examined: *Agnocardia dissidipictum* (Woodring).
- Trigoniocardia*, type species *Trigoniocardia granifera* (Broderip and Sowerby). Species examined: *Trigoniocardia antillarum* (d'Orbigny).
- Tridacna*, type species *Tridacna gigas* (Linnaeus). Species examined: *Tridacna maxima* (Röding).
- Hypanis*, type species *Hypanis plicatum* (Eichwald). Species examined: *Hypanis colorata* (Eichwald).

Preliminary phylogenetic analysis of the bivalve family Galeommatidae

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Abstract. A preliminary phylogenetic analysis of species assigned to the ill-defined family Galeommatidae, plus selected others, was attempted in an effort to clarify the relative value of various systematic characters. Aspects of dealing with large numbers of equally parsimonious trees in cladistic analyses and intrinsic problems of analyses based on unordered multistate characters are addressed briefly. The analysis (Hennig86, implicit enumeration) of 18 characters with 46 character states for 20 species yielded 164 equally parsimonious trees (length 52, ci 53, ri 72), displaying five distinctly different branching patterns. Separate consensus trees were produced for the five groups. Of the five topologies, one is considered most likely in an evolutionary context, and is discussed in detail. Three consistent species groups were recognized: 1) *Divariscintilla* group (eight species); 2) *Galeomma-Ehippodonta* group (six species); and 3) *Scintillona-Ceratobornia* group (three species). Two specialized anatomical characters were analyzed for relative systematic value: 1) "hanging" foot morphology, which could have evolved more than once within the Galeommatoidea; 2) flower-like organs, a possible synapomorphy of the *Divariscintilla* species-group. Results also indicated that the monospecific *Phlyctaenachlamys* Popham, 1939, is a junior subjective synonym of *Divariscintilla* Powell, 1932, and suggested that the generic limits of *Galeomma* Sowerby In: Turton, 1825, and *Ehippodonta* Tate, 1889, should be reexamined. Data matrix construction further identified potentially valuable, but currently unusable, characters in need of further investigation: occurrence of flower-like organs; foot morphology including byssus gland(s); homologies of hinge teeth; ligament/resilium apparatus; shell microstructure; reduction/loss of ctenidial interlamellar junctions and outer demibranch; presence/extent of midgut typhlosole, innervation of pallial tentacles, and sperm structure.

"The interchanges of characters and the multiplicity of forms separated by apparently trifling details of structure make this group one of the most perplexing I have ever tried to review."
— William H. Dall, 1899:875.

Galeommatoidean bivalves are recognized by a suite of character specializations, character reductions and possible cases of convergence (Boss, 1965; Morton and Scott, 1989). Common traits include a muscular foot modified for snail-like locomotion, a byssus gland present in the adult, species-specific arrangements of sensory papillae and tentacles, anterior-to-posterior water flow through the mantle cavity, and eulamellibranch ctenidia. Trends within the group are toward shell reduction, with corresponding reductions in sculpture, hinge structure and adductor muscles, internalization of the shell by mantle lobes, reduction of the outer demibranch, commensalism, and reproductive specialization, including hermaphroditism, brooding, dwarf males (B. Morton, 1976, 1981; Ó Foighil, 1985a), spermatophores (Ó Foighil, 1985a, b), and mating behavior (Mikkelsen and Bieler, in press).

Five species in the galeommatid genus *Divariscintilla* have been described recently from eastern Florida (Mikkelsen and Bieler, 1989, in press). All five are co-occurring commensals with a single species of burrowing mantis shrimp [*Lysiosquilla scabricauda* (Lamarck)] that inhabits shallow-water sand flats. The only other known member of the genus is the type species, *D. maoria* Powell, 1932, also commensal

in mantis shrimp burrows, in New Zealand.

Anatomical description of the five Floridian species has resulted in redescription of the genus (Mikkelsen and Bieler, 1989), incorporating an interesting suite of complex characters reflective of the clams' specialized nature and habitat. Two of these characters ("hanging" foot morphology, flower-like organs, discussed below), although diagnostic of the genus, are also known from species assigned to other genera, sometimes placed in other nominal families, of Galeommatoidea.

"Hanging" foot morphology involves a bipartite foot with a muscular anterior crawling portion, and an elastic posterior extension. A ciliated ventral groove extends from the primary byssus gland in the antero-ventral part to the terminal, internally-lamellar byssus adhesive gland (see Mikkelsen and Bieler, 1989). This morphology is present in members of the five Floridian *Divariscintilla* species (Mikkelsen and Bieler, 1989: figs. 18, 19, 21, 22; in press), *D. maoria* (see Judd, 1971: 351-352, figs. 1-4; pers. obs.), and *Phlyctaenachlamys lysiosquillina* Popham, 1939 (: 64, figs. 1, 7). It is also known from three members of Lasaeidae (= Erycinidae): *Parabornia squillina* Boss, 1965 (: 4, fig. 3; pers. obs.); *Ceratobornia longipes* (Stimpson, 1855) (Dall, 1899: 889, pl. 88, figs. 10, 11, 13); *C. cema* Narchi, 1966 (: 515, figs. 1, 2, 5).

Flower-like organs are located on the anterior surface of the visceral mass, just ventral to the labial palps. They

are mushroom-shaped, without major nervous supply, with a "head" composed of onion-shaped secretory units emptying into the anterior pallial cavity (Mikkelsen and Bieler, 1989: fig. 23). Their number is species-specific and constant, except in *Divariscintilla yoyo* which has from three to seven organs in a close cluster. Their function is undetermined, but is probably pheromonal, related to intraspecific communication with potential reproductive partners or with free-swimming veliger larvae (Mikkelsen and Bieler, 1989, in press). Flower-like organs are present in members of four of the five Floridian *Divariscintilla* species (Mikkelsen and Bieler, 1989: figs. 26, 27; in press), *D. maoria* (see Judd, 1971: 352, figs. 2, 4, PP; pers. obs.), *Phlyctaenachlamys lysiosquillina* (two in number, pers. obs.), *Vasconiella jeffreysiana* (P. Fischer, 1873) (see Cornet, 1982: fig. 5), and the lasaeid *Parabornia squillina* (single, pers. obs.).

The family Galeommatidae is ill-defined at present. It is differentiated traditionally from other Galeommatoidae mainly by hinge teeth, generally described as irregular, edentulous, or with weak tubercular cardinals (e.g. Chavan *In*: Moore, 1969a; Kay, 1979). This reflects general reduction in the hinge, rather than any defined synapomorphy for the group. In this context, the systematic value of the two specialized characters relative to traditionally employed characters (e.g. hinge teeth, ctenidia) is of interest. In response, a phylogenetic analysis of species assigned to Galeommatidae, plus selected others, was attempted. Characters involving the shell, mantle, mantle cavity, reproduction, and ecology were included, and type species of genera were used whenever possible. The analysis is considered preliminary, in the sense that it is partially based on limited literature data, and as such does not propose to resolve phylogenetic relationships for the family. It has, however, shown the distribution of foot- and flower-like-organ-characters within the group, and perhaps more importantly, identified characters which could not be used at this time due to insufficient data.

MATERIALS AND METHODS

TAXA

Ingroup: Species were selected from as many galeommatid genera as possible, but dependent on those with adequate, available anatomical data. Type species were included whenever possible. Twenty species [18 in Galeommatidae, two in Lasaeidae (= Erycinidae)] were included as members of the ingroup (Appendix 1). Data for most non-Floridian species were based on published literature, but in several cases were supplemented or verified by original observations of specimens (Appendix 1).

Outgroup: Out initial attempts used either or both of the galeommatoid (but presumably non-galeommatid) species *Montacuta substriata* (Montagu, 1808) and *Lasaea*

rubra (Montagu, 1803) as outgroups. This was abandoned when it became clear that these taxa, with their own specializations (and unresolved taxonomic questions) introduced additional homoplasy complicating the efforts to estimate in-group relationships. Instead, a hypothetical bivalve (all character states = 0) was used as the outgroup. This bivalve, as defined by our character set, approximates closely a generalized member of Lucinoidea, e.g. *Lucina* (see Chavan *In*: Moore, 1969b), except in morphology of the foot which in lucinid species is specialized for burrowing (Yonge and Thompson, 1976).

Abbreviations of repositories are as follows: AMS, Australian Museum, Sydney; BMNH, Natural History Museum, London; CAS, California Academy of Sciences, San Francisco; FSBC, Florida Marine Research Institute, Department of Natural Resources, St. Petersburg; NMP, Natal Museum, Pietermaritzburg, South Africa.

CHARACTERS

Characters used in the analysis were dependent upon those features that could be adequately coded from statements in the literature. Particular attention was paid to those characters which have been employed in genus- and family-level descriptions, e.g. hinge structure. Additional characters were evaluated but could not be used (see Discussion). Eighteen characters were used, involving the shell (ten), anatomy (six), reproduction (one), and life habit (one) (Appendix 2). Commensalism was superimposed upon the completed trees to visualize the taxonomic distribution of commensal versus non-commensal species.

Hinge characters comprised five of the ten shell characters used in this analysis, yet they proved exceedingly difficult to code with regard to the nature and numbers of teeth present (see Discussion). In the absence of data concerning tooth homologies, we coded functional presence/absence states for both cardinal and lateral teeth (characters 5 and 6), i.e. present and interlocking, or present and noninterlocking, or absent. The location of both lateral teeth (character 7), either anterior, posterior or both, was also coded. Thickened ridges along the hinge line (character 8) in several species could not be interpreted as modified lateral teeth with confidence, so they were coded separately as present/absent.

The extent of mantle coverage over the shell (character 10) could not be determined reliably from preserved material nor from published reports based on preserved material alone. Therefore, one question-mark (for *Vasconiella jeffreysiana*) exists in the coding of this character in the final data set. Interpreting the degree of internalization was also a problem. Some species descriptions indicated complete internalization (e.g. *Coleoconcha opalina*, see Barnard, 1964). However in two species with such descriptions (*Chlamydoconcha orcutti*, see B. Morton, 1981; *Phlyctaenachlamys lysiosquillina*, see

Popham, 1939), an umbonal foramen actually exists connecting the external environment and the cavity containing the shell (B. Morton, 1981, and pers. obs., respectively). The mantle tissue with this type of opening is not retractable, even upon preservation. These two cases show a higher degree of mantle fusion than that seen in, e.g. *Divariscintilla troglodytes*, where retraction exposes more than half of the shell. However, in view of the difficulties experienced with *C. orcutti* and *P. lysiosquillina*, all cases of mantle fusion preventing complete retraction, regardless of the degree, were coded identically.

The character "dymantic tentacles" refers to two single dorsal tentacles, one anterior and one posterior, which are used in dymantic, or defensive, display (see B. Morton, 1975). They were coded as present when present morphologically, even when dymantic behavior had not been documented (e.g. for *Galeomma turtoni*, see Popham, 1940). They were coded separately from other, non-dymantic tentacles, which usually exist in lateral pairs.

ANALYSIS

The final data matrix appears in Appendix 3. The Hennig86 program package (version 1.5; Farris, 1988) was used for this analysis on a 486-class IBM-compatible personal computer. Tree generation utilized "implicit enumeration" (ie), an algorithm that guarantees finding all shortest equally parsimonious trees. The terms *consistency index* (ci) and *retention index* (ri) are employed as defined by Kluge and Farris (1969) and Farris (1989), respectively.

No *a priori* assumptions were made regarding

character importance (weighting) or evolutionary direction (ordering of multistate characters). The use of unordered character states avoids bias in tree development. However, the algorithm can find it more parsimonious to interpret the (initially presumed plesiomorphic) state of the outgroup as autapomorphic and the initially presumed synapomorphic state of the ingroup as a symplesiomorphy, shared with the hypothetical ancestor of both ingroup and outgroup. It is therefore necessary to scrutinize every resulting tree for this occurrence [e.g. as discussed further below, all resulting trees assumed some extent of mantle coverage (character 10) for the hypothetical ancestor].

RESULTS

Based on the rigorous ie-algorithm, 164 equally parsimonious trees (length 52, ci 53, ri 72) resulted from the analysis. Each tree was analyzed and most (96%) could be assigned to one of five distinct tree topologies. The remaining trees (4%) were combinations of the five scenarios.

Four of the five topologies (56% of the trees generated) are here considered less likely in an evolutionary context because they are based on assumptions such as complete shell coverage by the mantle in the hypothetical ancestor (character 10 state 3), reversal from lost to interlocking lateral hinge teeth (character 6), a flattened limpet-like ancestor (character 17), or a large number of character state reversals (as opposed to parallel acquisition). The major species groups (discussed below) in these trees were recognizable but often as grades rather than clades.

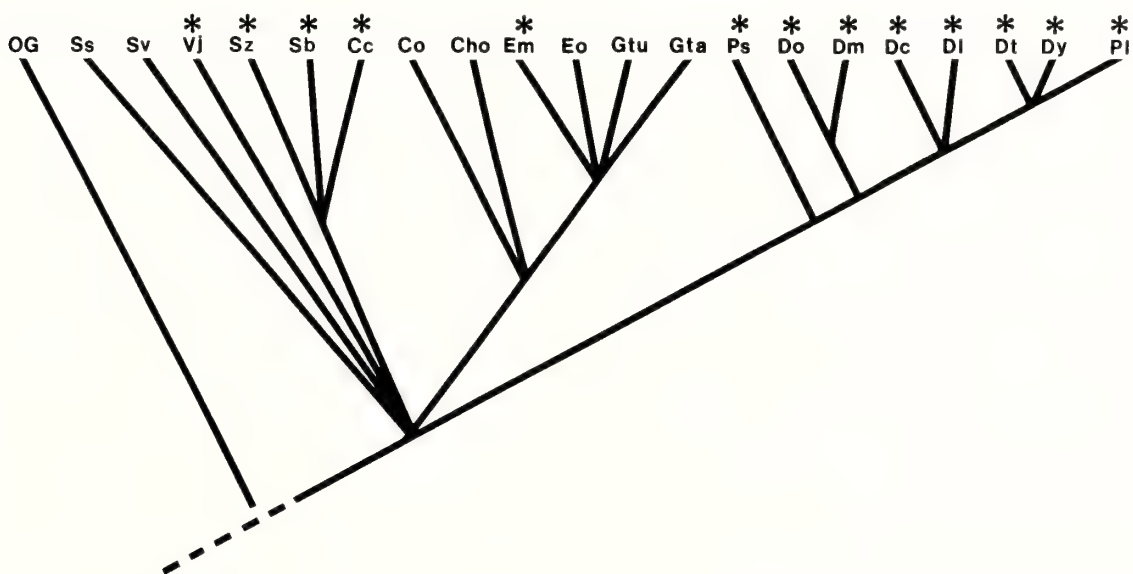


Fig. 1. Nelson (strict) consensus tree of the one of five tree topologies that is here considered most likely in an evolutionary context (based on 44% of all trees generated) [Length = 52, consistency index (ci) = 53, retention index (ri) = 72, * = species known to live commensally with another invertebrate species, OG = hypothetical bivalve outgroup (for other acronyms see Appendix 1)].

The Nelson (strict) consensus tree for the fifth topology, here considered most likely in an evolutionary context (within the limits of the current dataset), is presented in figure 1. Three monophyletic groups were distinct: 1) *Divariscintilla* group (eight species), including *Phlyctaenachlamys lysiosquillina* (Pl), with *Parabornia squillina* (Ps) forming the sister group to *Divariscintilla*; 2) *Galeomma-Ehippodonta* group (six species), including *Coleoconcha opalina* (Co) and *Chlamydoconcha orcutti* (Cho); 3) *Scintillona-Ceratobornia* group (three species), comprising *Scintillona zelandica* (Sz), *S. bellerophon* (Sb), and *Ceratobornia cema* (Cc). *Scintilla stvensoni* (Ss), *S. violescens* (Sv), and *Vasconiella jeffreysiana* (Vj) were not affiliated clearly with any of the three groups, but the two *Scintilla* species (Ss, Sv) appear to be *not* monophyletic. The relationships between the three major branches remain unresolved.

Within the *Divariscintilla*-group, several stable subunits can be recognized. Based on the common character state of anterior shell prolongation (character 2), Dy, Dt and Pl group together. The taxa Do and Dm are linked because of their low degree of shell coverage by the mantle (character 10). Five species (Dc, Dl, Dt, Dy and Pl) fall together because of shell reduction (character 0). *Parabornia squillina* (Ps) joins this clade because of the elongated foot (character 4), absence of lateral hinge teeth (character 7) and presence of a flower-like organ (character 13).

The species of the *Galeomma-Ehippodonta*-group always group together based on the synapomorphies of lateral hinge ridges (character 8), beaded shell sculpture (character 9) and dimantic tentacles (character 12), but with equivocal distinction between the two nominal genera. Two additional taxa (Co and Cho) join the clade based on overall limpet-shape (character 17; Co only), presence of dwarf males (character 16), and a number of character state losses (e.g. cardinal and lateral hinge teeth, characters 5 and 6).

The clade of the *Scintillona-Ceratobornia*-group is determined by the indented hinge plate (character 4), non-interlocking cardinal hinge teeth (character 5), the low degree of shell coverage by the mantle (character 10), and by the specialized foot morphology (character 14, *Scintillona* spp. only).

Two characters in the analysis were interpreted consistently as autapomorphies and not synapomorphies: inequivalve shells and ventral shell notch (characters 1 and 3). The state changes of two other characters (7 and 15; lateral teeth position and adductor muscles) could not positively be placed and could have happened in any of several branches of the trees.

"Hanging" foot morphology (character 14) was not confined to a single group, and may therefore be convergent. It does appear in most members of the *Divariscintilla* group, but also in *Ceratobornia cema* (Cc). Flower-like organs were

primarily confined to members of the *Divariscintilla* group, but are also present in *Vasconiella* (Vj), a taxon of uncertain affiliation at this point. Commensal species (Fig. 1, *) are distributed widely on all trees, confirming a trend toward commensalism in the superfamily, but not defining any taxonomic group.

DISCUSSION

This analysis, that could *not* employ a number of recognizably valuable characters (see below), and that resulted in such a high number of equally parsimonious trees, is obviously a preliminary one. However, without over-interpreting the results, five generalizations can be made. 1) The described *Divariscintilla* species and monotypic *Phlyctaenachlamys* appear to form a monophyletic group. If treated as one genus, *Divariscintilla* Powell, 1932, has priority over *Phlyctaenachlamys* Popham, 1939. 2) The generic allocation of species in *Galeomma* versus *Ehippodonta* needs further study. 3) The postulated close relationship (same genus) of *Scintilla stvensoni* and *S. violescens* is questionable. 4) The relationships between *Ceratobornia cema* and the two studied *Scintillona* species also warrant further investigation. 5) The relationship between *Parabornia squillina* and *Divariscintilla* species, usually placed in different nominal families, needs additional study.

Improvement on the data set using more specimen- (versus literature-) based data is of course indicated before strong taxonomic decisions can be made as a result of phylogenetic analysis of this group. Character states are too often ambiguous in written descriptions, even more so if interpreted from line drawings or photographs. Furthermore, an appreciation of variability of characters within a species (see below) is seldom available in the literature.

We assume that the complex "hanging" foot morphology (with associated glandular structures) and flower-like organs represent synapomorphies within Galeommatodea, but the extent of the groups defined by them presently remains unclear.

Perhaps the most valuable result of this study was the identification of numerous characters that, although potentially valuable, could not be used in the analysis due to lack of data on species not studied by us. These are discussed below as suggestions for needed comparative investigations and/or as useful items to include in future species descriptions.

1. The occurrence of both specialized characters emphasized in this study ("hanging" foot morphology, flower-like organs) requires additional documentation. Examination of additional *Phlyctaenachlamys lysiosquillina* specimens during this study suggested that flower-like organs may vary within a species. These organs were not mentioned or indicated in drawings of anatomy or histological sections in

the excellent original description by Popham (1939). Nor were they present in one of Popham's paratypes (BMNH 1939.5.10.2) examined by us. Therefore, we trust that flower-like organs were not merely overlooked by Popham. Nevertheless, each of three specimens from the Australian Museum (C165143) had two well-defined flower-like organs. Whether this reflects populational, reproductive or seasonal variation is unknown.

In addition to flower-like organs, two other features of *Phlyctaenachlamys lysiosquillina* were clarified during examination of the Australian Museum specimens, and are worthy of mention here: 1) the existence of an umbonal foramen (see above); 2) the presence of ctenidial interlamellar junctions (in inner demibranch, approximately mid-gill) which had been stated as absent by Popham (1939: 72).

2. Hanging foot morphology is complicated. Oldfield (1955, 1961) showed multiple byssus glands in various galeommatoideans which compliment our findings in *Divariscintilla*. In *Lasaea rubra*, a "subsidiary byssus gland" empties into the canal of the main byssus gland with its associated byssogenous lamellae in the posterior "heel" of the foot (Oldfield, 1955: 233-234, fig. 4, BG1, BG2, BL). In *Montacuta substriata*, several subsidiary glands "open by long, slender ducts, into the extreme anterior end of the byssus [ventral] groove" (Oldfield, 1961: 270, fig. 7, BG1-3, BL), mirroring the condition seen in Floridian *Divariscintilla* species. In light of these data, as mentioned previously (Mikkelsen and Bieler, 1989), the anteroventral "mucous gland" in the two-part foot of *Ceratobornia cema* should be reevaluated. Behavioral observations and histochemical techniques would be valuable in this area.

3. As was implied above, galeommatoidean hinge teeth are difficult to interpret. Problems such as small subumbonal tubercles which may or may not interlock, or lateral ridges which may or may not be true lateral teeth, are not uncommon. Unresolved questions of this kind involving tooth homology prevented rigorous coding of the hinge teeth, and will require ontogenetic studies to resolve with certainty.

Also concerning the hinge, the external ligament and resilium have been described in a variety of ways, e.g. with or without nymph/resilifer/socket, triangular or oblique, subumbonal between teeth or posterior (for examples, see Chavan *In*: Moore, 1969a). Whether these are real differences or mere variation in wording must await reanalysis of hinge structures.

4. Coney (1990) and ourselves (Mikkelsen and Bieler, 1989) have illustrated and described shell microstructure in several galeommatoidean species. Additional species should be investigated using more consistent and rigorous methods.

5. Second only to the hinge in traditional taxonomic use in Galeommatoidea are the ctenidia. Two potentially useful characters could perhaps be quantified for use in an analysis such as this. 1) Relative size of the outer demibranch

has been subjectively recorded but with implied quantifiable differences, e.g. "much shorter" (*Scintillona zelandica*; J. E. Morton, 1957: 185), "slightly [reduced]" (*Ephippodonta maddougalli*; Woodward, 1893), "longer, dorso-ventrally than the inner" (*Chlamydoconcha orcutti*; B. Morton, 1981). Complete loss of the outer demibranch is characteristic of Montacutidae. 2) Reduction in number or complete loss of interlamellar junctions have been correlated with extensive expansion/contraction of the mantle (Popham, 1939; Narchi, 1966), or with the incubation of larvae in the suprabranchial chamber (B. Morton, 1981). Four of the five Floridian *Divariscintilla* species are known to brood and to have interlamellar junctions. The presence of interlamellar junctions in the type species, *D. maoria*, was also confirmed during this study (from Australian Museum specimens, collected by W. Judd, C165142).

6. The presence and extent of a midgut typhlosole appear to differ among species. For example, it has been reported as absent in *Ceratobornia cema* (see Narchi, 1966), *Phlyctaenachlamys lysiosquillina* (see Popham, 1939), and *Montacuta* spp. (Oldfield, 1961), and as present in *Chlamydoconcha orcutti* (see B. Morton, 1981), *Divariscintilla* spp. (Mikkelsen and Bieler, 1989, in press), and *Galeomma takii* (see B. Morton, 1973).

7. Innervation of the various pallial tentacles could reveal patterns and possible homologies. Many species (e.g. *Ceratobornia cema*, *Scintilla violescens*, *Galeomma turtoni*) show unpaired tentacles along the dorsal midline; in *Divariscintilla yoyo*, these are known to be innervated by branches from both pallial nerves (Mikkelsen and Bieler, 1989: fig. 31). The innervation of "dymantic" tentacles (B. Morton, 1975, 1976) in *Galeomma* and *Ephippodonta* species is especially important to this line of inquiry.

8. Finally, in this group where reproductive complexity is the rule, sperm structure could be a conservative, valuable indicator of phylogenetic relationships. Reported morphologies include elongated curved heads with collared acrosomes [*Divariscintilla* spp., Eckelbarger *et al.*, 1990; *Lasaea australis* (Lamarck, 1818), Ó Foighil, 1988], elongated straight heads with cone-shaped acrosomes [*Mysella tumida* (Carpenter, 1864), Ó Foighil, 1985b], and oval heads (*Chlamydoconcha orcutti*, B. Morton, 1981).

ACKNOWLEDGMENTS

We thank Terrence Gosliner (CAS), Richard N. Kilburn (NMP), William G. Lyons (FSBC), Winston F. Ponder (AMS), and Kathie Way (BMNH) for the loan of specimens. Walter Sage (American Museum of Natural History, New York) and Paul Scott (Santa Barbara Museum of Natural History, California) provided needed literature. Two anonymous reviewers are thanked for their comments. The research was supported in part by the Smithsonian Marine Station at Link Port; the cooperation of Dr. Mary Rice and her staff is gratefully acknowledged. This is Harbor Branch Oceanographic Institution Contribution No. 878 and Smithsonian Marine Station Contribution No. 290.

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Date of manuscript acceptance: 21 October 1991

APPENDIX 1.

Taxa included in the analysis. Reference(s) are sources of literature or original (pers. obs.) data (T = type species of the genus; M = type species of monotypic genus).

	Reference(s)
GALEOMMATIDAE (18)	
M <i>Chlamydoconcha orcutti</i> Dall, 1884 (Cho)	Bernard, 1897; Chavan <i>In</i> : Moore, 1969a; B. Morton, 1981.
M <i>Coleoconcha opalina</i> Barnard, 1964 (Co)	Barnard, 1964; pers. obs. (NMP A1747).
T <i>Divariscintilla maoria</i> Powell, 1932 (Dm)	Powell, 1932, 1979; Judd, 1971; Coney, 1990; pers. obs. (AMS C165142).
<i>D. troglodytes</i> Mikkelsen and Bieler, 1989 (Dt)	Mikkelsen and Bieler, 1989.
<i>D. yoyo</i> Mikkelsen and Bieler, 1989 (Dy)	Mikkelsen and Bieler, 1989.
<i>D. n. sp. "heart-shaped"</i> (Dc)	Mikkelsen and Bieler, in press.
<i>D. n. sp. "yellow"</i> (DI)	Mikkelsen and Bieler, in press.
<i>D. n. sp. "white"</i> (Do)	Mikkelsen and Bieler, in press.
<i>Ehippodonta macdougalli</i> Tate, 1889 (Em)	Tate, 1889; Woodward, 1893; Chavan <i>In</i> : Moore, 1969a; pers. obs. (CAS 077807).
<i>E. oedipus</i> B. Morton, 1976 (Eo)	B. Morton, 1976.
T <i>Galeomma turtoni</i> Turton, 1825 (Gtu)	Mittre, 1847; Popham, 1940; Tebble, 1966; Chavan <i>In</i> : Moore, 1969a; B. Morton, 1973; Angloy, 1988; pers. obs. (CAS 41198).
<i>G. takii</i> (Kuroda, 1945) (Gta)	Kuroda, 1945; B. Morton, 1973; B. Morton and Scott, 1989.
M <i>Phlyctaenachlamys lysiosquillina</i> Popham, 1939 (Pl)	Popham, 1939; Coney, 1990; pers. obs. (AMS C165143).
<i>Scintilla stevensoni</i> Powell, 1932 (Ss)	Powell, 1932, 1979; Ponder, 1967.
<i>S. violescens</i> Kuroda and Taki, 1961 (Sv)	Arakawa, 1961; Kuroda and Taki, 1961; B. Morton, 1976.
T <i>Scintillona zelandica</i> (Odhner, 1924) (Sz)	Odhner, 1924; J. E. Morton, 1957; Chavan <i>In</i> : Moore, 1969a.
<i>S. bellerophon</i> Ó Foighil and Gibson, 1984 (Sb)	Ó Foighil and Gibson, 1984.
M <i>Vasconiella jeffreysiana</i> (P. Fischer, 1873) (Vj)	Kisch, 1958; Cornet, 1982; Coney, 1990.
LASAEIDAE (2)	
<i>Ceratobornia cema</i> Narchi, 1966 (Cc)	Narchi, 1966.
M <i>Parabornia squillina</i> Boss, 1965 (Ps)	Boss, 1965; pers. obs. (FSBC I6943).

APPENDIX 2.

Characters and character states used in the phylogenetic analysis.

SHELL

- Size relative to mantle.
0 = subequal, 1 = significantly smaller than mantle.
- Size of valves relative to each other.
0 = equivalve, 1 = inequivalve.
- Prolongation.
0 = equilateral, 1 = anteriorly, 2 = posteriorly.
- Ventral notch.
0 = absent, 1 = notched in one valve, 2 = notched in both valves.
- Hinge plate.
0 = not indented, 1 = indented.
- Hinge - cardinal teeth.
0 = interlocking, 1 = present, not interlocking, 2 = absent.
- Hinge - lateral teeth.
0 = interlocking, 1 = present, not interlocking, 2 = absent.
- Hinge - lateral teeth (position).
0 = anterior + posterior, 1 = anterior only, 2 = posterior only.
- Hinge - lateral thickened ridges.
0 = absent, 1 = present.
- Sculpture.
0 = not reticulate/beaded, 1 = reticulate/beaded.

ANATOMY

- Extent of mantle coverage.
0 = none, 1 = margins only, 2 = complete, but retractable, 3 = complete, with fusion preventing complete retraction.
- Elongated tentacles (excluding dymantic tentacles).
0 = absent, 1 = present.
- Dymantic tentacles.
0 = absent, 1 = present.
- Flower-like organs.
0 = absent, 1 = present.
- Foot structure.
0 = cylindrical anterior + blunt heel, 1 = cylindrical anterior + elongated heel, 2 = blade-like anterior, without distinct heel.
- Adductor muscles.
0 = subequal, 1 = posterior reduced, 2 = anterior reduced, 3 = both absent.

OTHER

- Reproductive - dwarf male.
0 = absent, 1 = present.
- Life habit.
0 = not flattened limpet-like, 1 = flattened limpet-like.

APPENDIX 3.

Data matrix (? = character state unknown; - = character state not applicable;
OG = hypothetical bivalve outgroup; for other acronyms see Appendix 1).

CHARACTERS

Taxa	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7
OG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Co	0	0	2	0	0	2	2	-	0	0	3	0	0	0	0	3	1	1
Dm	0	0	2	2	0	1	1	2	0	0	1	1	0	1	1	0	?	0
Dc	1	0	2	2	0	1	2	-	0	0	2	1	0	1	1	0	0	0
Dl	1	0	2	0	0	1	2	-	0	0	2	1	0	1	1	0	?	0
Do	0	0	2	0	0	1	2	-	0	0	1	1	0	0	1	0	0	0
Dt	1	0	1	0	0	1	2	-	0	0	3	1	0	1	1	0	0	0
Dy	1	0	1	0	0	1	2	-	0	0	3	1	0	1	1	0	0	0
Em	0	0	0	0	0	1	2	-	1	1	3	1	?	0	0	1	0	1
Eo	0	0	0	0	0	2	2	-	1	1	2	0	1	?	?	?	?	1
Gtu	0	0	2	0	0	2	2	-	1	1	3	0	1	0	0	2	1	1
Gta	0	0	2	0	0	0	2	-	1	1	3	0	1	0	0	0	?	1
Pl	1	0	1	0	0	1	0	2	0	0	3	1	0	1	1	1	0	0
Ss	0	0	0	0	0	0	0	1	0	0	3	0	0	0	1	0	?	1
Sv	0	0	0	0	0	0	0	1	0	0	2	1	0	?	0	?	?	0
Sz	0	0	0	0	1	1	0	0	0	0	1	0	0	0	2	0	?	0
Sb	0	0	0	0	1	1	0	1	0	0	1	1	0	0	2	0	0	0
Vj	0	1	0	1	0	0	0	2	0	0	?	1	0	1	0	0	?	0
Ps	0	0	2	0	0	0	2	-	0	0	2	1	0	1	1	0	?	0
Cho	1	1	1	0	0	2	2	-	0	0	3	0	0	0	0	1	1	0
Cc	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	?	0

Seasonal variations in brood size of *Lasaea* cf. *nipponica* (Bivalvia: Galeommatoidea) in Hong Kong

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Abstract. Monthly samples of *Septifer virgatus* Weigmann, 1837 (Mytilidae) from an exposed rocky shore in Hong Kong were examined for *Lasaea* cf. *nipponica*. Evidence for assigning this name to the Hong Kong species of *Lasaea* is given.

Lasaea cf. *nipponica* is hermaphroditic and produces two broods of crawl-away juveniles each year, in spring (May) and autumn (October to December). Although brood size is a function of parental size, spring parents brood large numbers ($\bar{x} = 81.5/\text{parent}$) of larger ($\sim 320\text{--}330\ \mu\text{m}$ shell length) juveniles; autumn parents brood fewer ($\bar{x} = 24/\text{parent}$) numbers of smaller ($\sim 300\text{--}320\ \mu\text{m}$ shell length) juveniles. Greater variation in the size of juveniles comprising the spring broods, however, suggests that a seasonal comparison of this factor could be non-significant and requires further study.

This study suggests that variations in brood size (and possibly juvenile size) results from seasonal differences in environmental factors acting upon the parents. Brood size (and possibly juvenile size) is therefore likely to vary not just seasonally, but across the spectrum of populations encompassed by the species range.

Morton and Scott (1989) reported upon a species of *Lasaea* from Hong Kong shores and to which they ascribed the name *Lasaea rubra* (Montagu, 1803). Such a designation for a species with a presumed North Atlantic Ocean distribution (Keen, 1983) could be considered intrepid, except that Ponder (1971) believes the species to have a much wider distribution, encompassing the Pacific Ocean, than hitherto presumed, possibly because of artificial introductions. Regional workers, e.g. Habe (1977), and researchers of the Chinese marine Mollusca, e.g. Bernard *et al.* (in press), refer to the Asian species of *Lasaea* as *L. undulata* (Gould, 1861). Morton and Scott (1989) pointed out, however, that the Hong Kong species does not possess the prominent, widely spaced, concentric rings that are believed typical of *L. undulata*. Ó Foighil (1988) has, however, suggested that the presence or absence of heavy concentric rings is a poor character for separating species of *Lasaea*. The taxonomic status of the Hong Kong species of *Lasaea* will be discussed.

The Hong Kong species of *Lasaea*, i.e. *Lasaea* cf. *nipponica*, occurs amongst an $\sim 1\text{ mm}$ wide band of mussels (*Septifer virgatus* Weigmann, 1837) colonizing the area of mean high water neap tide (MHWNT) on exposed rocky shores. From September 1989 to August 1991, monthly samples of *S. virgatus* were inspected for *Lasaea*. These were, in turn, examined for the presence of brooded juveniles, with a view to: (a) obtaining characters which would aid in the identification of the local species of *Lasaea*; (b) reveal the pattern of reproduction and, thus, brooding undertaken by the species locally; (c) correlate features of brood and

juvenile sizes with adult size to assess the extent of intraspecific variation in this species. Coincidentally, hydrological parameters were investigated so as to provide a measure of the environmental changes the local species of *Lasaea* experiences and possible correlations with such important activities as gametogenesis and the release of crawl-away juveniles.

MATERIALS AND METHODS

Each month, between September 1989 and August 1990, samples of *Lasaea* cf. *nipponica* were obtained from standard (20 x 20 cm) random samples of *Septifer virgatus* along the exposed shores at Cape d'Aguilar, Hong Kong Island. The samples were fixed immediately in 5% neutral formalin. Each individual in each sample was measured along its greatest length to the nearest 0.1 mm using a dissecting microscope and eye-piece graticule. Wherever possible, ten individuals from each 0.1 mm size class so obtained were dissected and the ctenidia inspected for brooded juveniles. When found, they were isolated from each ctenidium and counted. Each one was then measured along its greatest length to the nearest 1 μm using a compound microscope and eye-piece graticule. Numbers of juveniles in the left and right ctenidia were separated, initially, but a subsequent test for differences showed there to be none and the two sets of information were pooled subsequently to give a total figure for the numbers of juveniles/parent. The results obtained will be discussed in relation to the prevailing hydrological climate

of Cape d'Aguilar and which was assessed by similarly monthly-obtained water samples that were analyzed in terms of temperature, salinity, pH and dissolved oxygen using standard procedures.

TAXONOMY

Bernard *et al.* (in press) record *Lasaea undulata* Gould, 1861 from the coast of China. This is considered to be the senior synonym for both *L. nipponica* Keen, 1938 and *Kellia minutissima* Habe, 1960 (Habe, 1977). Morton and Scott (1989), however, reported upon *L. rubra* (Montagu, 1803) from Hong Kong, noting the lack of strong concentric rings in local specimens but which are, apparently, characteristic of *L. undulata* (Keen, 1938). As this study will later show, however, the Hong Kong *Lasaea* is quite unlike *L. rubra* in terms of breeding season and brood size. Ó Foighil (1989) has suggested that the Hong Kong species of *Lasaea* produces crawl-away juveniles; this study will confirm that observation. Ó Foighil (1989) further points out that the species of *Lasaea* found in Kagoshima, Japan, referred to as *L. undulata*, produces planktotrophic larvae and appears to be identical, in terms of valve morphology, to the similarly planktotrophic Australian species *L. australis* (Lamarck, 1818). Ó Foighil (1989) further points out that in Kagoshima, there appears to be two species of *Lasaea*, one with planktotrophic larvae (*L. undulata*?) and one with crawl-away juveniles. In Japan, Taiwan and Hong Kong, species of *Lasaea* also have crawl-away juveniles. Keen (1938) compared her species, *L. nipponica*, from Japan with two other species, also from Japan, i.e. *L. striata* Tokanuga, 1906 and *L. undulata*, and dismissed both as possible conspecifics, the former being far too big (9.5 mm shell length), the latter too heavily ringed concentrically. *L. undulata* with a maximum shell length of ~ 5 mm is also larger than *L. nipponica* (2.9

mm shell length). The largest individual of the Hong Kong species of *Lasaea* ever found is 4.0 mm long, reproductively mature parents falling between 2.1 - 3.6 mm in shell length. Thus, in terms of shell form and size and the degree of concentric ringing, the Hong Kong species is most like *L. nipponica*. If it is true, moreover, that *L. undulata* produces planktotrophic larvae, whereas the second widely distributed species in Japan (plus Taiwan) produces crawl-away juveniles, then it seems possible that this species, and the Hong Kong species, is *L. nipponica* and is distinct from *L. undulata* despite its synonymy with the former species by recent workers, e.g. Habe (1977). In the absence of good comparative data it has, however, been confused with *L. undulata*, the latter name eventually coming to gain widest coinage. For the above reasons, I refer here to the Hong Kong species as *Lasaea cf. nipponica*. Scanning electron micrographs of two paratypes of *L. nipponica* are illustrated in figures 1A and B (California Academy of Sciences Reg. No. 7227 and 7228, respectively) (Invertebrate Zoology Cat. No. 065920). These individuals were collected from Watanoha, Rikuzen, N.E. Matsushima, Honshu, Japan by S. Nomura. A scanning electron micrograph of an individual of *Lasaea cf. nipponica* from Cape d'Aguilar, Hong Kong is illustrated in figure 1C. They appear to be conspecific. Subsequent information on the reproductive biology of *L. cf. nipponica* in Hong Kong will, hopefully, provide for a better interpretation of this species and allow separation of it from others.

RESULTS

Figure 2 shows the numbers of individuals of *Lasaea cf. nipponica* obtained from the standard *Septifer virgatus* samples over the course of one year from September 1989 - August 1990. Two peaks are obvious, the first in January 1990, the second in May 1991, when densities were ~ 1750 m²

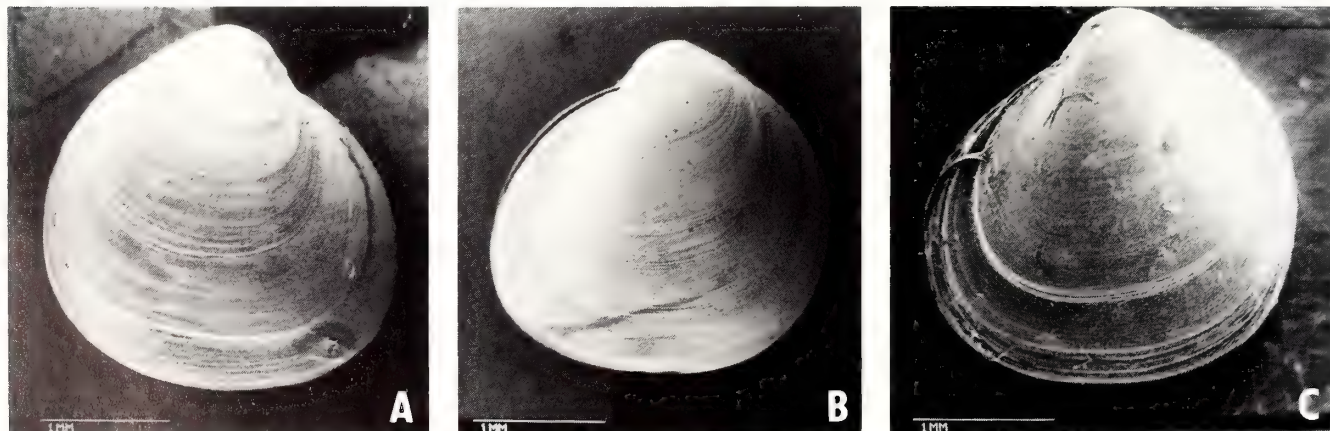


Fig. 1. A, B. Two paratypes of *Lasaea nipponica* (Keen, 1938) from Honshu, Japan (CAS Reg. No's. 7227 and 7228, respectively). C, *Lasaea cf. nipponica* from Cape d'Aguilar, Hong Kong.

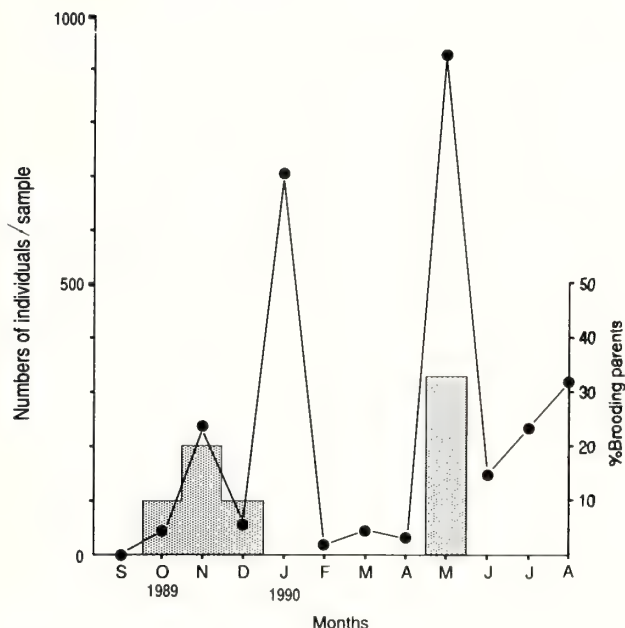


Fig. 2. *Lasaea* cf. *nipponica*. Numbers of individuals obtained in each monthly sample from *Septifer virgatus* beds at Cape d'Aguilar, Hong Kong (stippled areas indicate the % number of brooding parents in each sample).

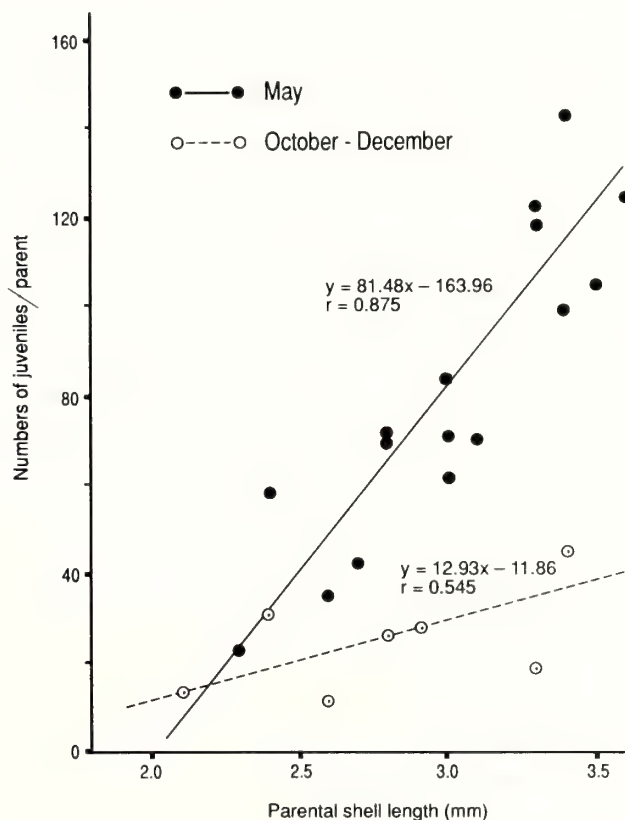


Fig. 3. *Lasaea* cf. *nipponica*. The numbers of juveniles/parent, expressed as a function of parental shell length, for both spring (May) and autumn (October - December) broods in Hong Kong.

and 2375 m^{-2} , respectively. At other times of the year, densities were low, all $< 600 m^{-2}$ and for most of the time $< 125 m^{-2}$. Overlay on figure 1 is the incidence of brooding individuals. Peaks in overall numbers in the population thus match peaks in the incidence of brooding. Juveniles were obtained from *L. cf. nipponica* parents in October, November and December 1989 and in May 1990, with the latter month providing the greatest % of brooding individuals, i.e. $> 36\%$.

Figure 3 compares the data obtained for parental shell length and numbers of juveniles/parent. As might be expected, brood size in both seasons is a function of parental size; bigger parents have bigger broods. From October to December, brooding individuals of up to 3.4 mm shell length were obtained with a mean of 24 shelled juveniles/parent and a maximum of 44 juveniles/parent, of 4.4 mm shell length. In May 1990, the picture was different, brooding individuals of up to 3.6 mm shell length were obtained with a mean of 81.5 juveniles/parent, although a maximum number of 151/parent, of 3.3 mm shell length, was recorded. Obtained values for brood sizes between the two periods have been tested for significance using an Analysis of Variance and are significantly different ($F = 19.835$; $P < 0.001$).

Figure 4 compares the data obtained for parental shell length (mm) and juvenile length (μm). In May 1990, average juvenile shell length, for each 0.1 mm increase in parental shell length, ranged between 308-335 μm . From October to December, values ranged between 298 and 323 μm . Juvenile shell length, at both times of the year, did not increase with

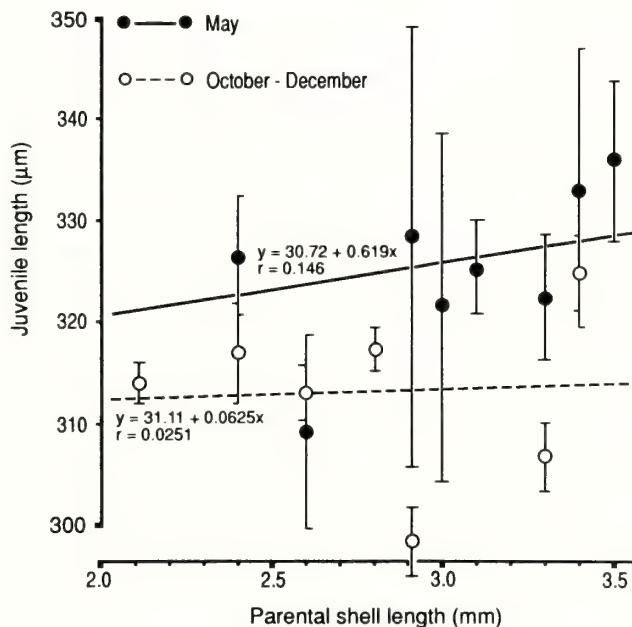


Fig. 4. *Lasaea* cf. *nipponica*. Juvenile shell length, expressed as a function of parental shell length, for both spring (May) and autumn (October - December) broods in Hong Kong (mean ± 1 S.D.).

parental shell length, i.e. bigger parents did not have bigger juveniles. Such data are significantly different ($P = 0.05$).

Figure 5 compares juvenile length (μm) and numbers of juveniles/parent. For May 1990, a mean juvenile length of $325 \mu\text{m}$ was obtained which did not vary significantly with respect to the numbers of juveniles present. From October to December, mean juvenile lengths varied between ~ 300 - $310 \mu\text{m}$, with an apparent decline in length with increasing numbers. This decline is not, however, statistically significant although it is true that the two sets of seasonal data are significantly different ($P = 0.05$).

The results obtained from this study suggest that spring parents (May) brood larger numbers ($\bar{x} = 81.5/\text{parent}$) of bigger juveniles (~ 320 - $330 \mu\text{m}$); autumn parents (October - December) brood fewer ($\bar{x} = 24/\text{parent}$), smaller (~ 300 - $320 \mu\text{m}$), juveniles. The large standard deviations in juvenile shell lengths (Figs. 3 and 4), however, cast doubt on the significant difference obtained for the data between the two seasons. Note, for example in figure 4, the wide standard deviations in juvenile shell lengths obtained for spring parents of 2.6, 2.9, 3.0 and 3.4 mm shell length. This would suggest that in May, a variety of developmental stages were present in the broods whereas from October - December, broods were more uniformly at a similar stage of development.

DISCUSSION

Species of the cryptic galeommatoidean genus *Lasaea*, occupy the high intertidal virtually world-wide and have aroused interest because of their smallness ($< \sim 5 \text{ mm}$), their adaptations to such a high-zoned life (Ballantine and Morton, 1956; Morton, 1956, 1960; Morton *et al.*, 1957) and their mode of reproduction, i.e. simultaneous herma-

phroditism and self-fertilization, with ctenidial brooding (Oldfield, 1955; Ó Foighil, 1987; Ó Foighil and Eernisse, 1988). Seed and O'Connor (1980) followed seasonal changes in population structure of *Lasaea rubra* on the north-east coast of Ireland and suggested that the population comprised three to four age groups, but with few individuals living beyond their third year. McGrath and Ó Foighil (1986) also studied the population dynamics of *L. rubra* in south-east Ireland and showed essentially the same pattern, i.e. the polymodal population picture suggested a life span of 2-3 years, with only a few individuals entering their fourth year.

The work of Oldfield (1964), Seed and O'Connor (1980) and McGrath and Ó Foighil (1986) all suggest that *Lasaea rubra* in the British Isles reproduces in a single phase over summer, typically from May to October, with maximum numbers of brooded, crawl-away, juveniles occurring from June-July and with peak recruitment occurring in August. Oldfield (1964) studied the life cycle of *L. rubra* in south-west England and estimated an incubation period of about two months. McGrath and Ó Foighil (1986) have summarized the literature on reproduction in *L. rubra* and of other species, notably *L. subviridis* from the north-east Pacific. The latter species broods juveniles year round but with peaks occurring at different times of the year, according to location. *L. rubra hinemoa* (from New Zealand) and *L. australis* (from Australia) similarly brood juveniles year round (Booth, 1979; Roberts, 1984).

Table 1 summarizes the data on the above species of *Lasaea* and compares it with information obtained about the species reported upon in this study. The Hong Kong species of *Lasaea* shows some similarities with other species, e.g. the maximum size of brooding adults (3.6 mm) approximates that recorded for *L. subviridis* (Glynn, 1965; Ó Foighil, 1985; Beauchamp, 1986). In other respects, however, notably with regard to the juveniles, the species is distinct, i.e. brood size ranges from means of 24 - 81.5/parent, in autumn and spring, respectively, whereas for other species it is far fewer. Similarly, crawl-away juvenile length is approximately half that of the other species hitherto investigated. Moreover, the local species broods juveniles in two phases, i.e. spring and autumn, whereas *L. subviridis* broods juveniles year round (Ó Foighil, 1985) and *L. rubra*, in the north-eastern Atlantic, is a summer brooder (Oldfield, 1955, 1964; Seed and O'Connor, 1980; McGrath and Ó Foighil, 1986). *L. rubra* does, however, brood over the same time frame as *L. cf. nipponica* in Hong Kong, i.e. from May - $\sim$ November.

Russell and Huelsenbeck (1989) report upon variations in brood size and brood structure in the small venerid bivalve *Transenella confusa* Gray, 1982, from California. In this species, although broods are present in some individuals throughout the year, brood size was significantly lower in winter although it is also a function of adult length (as shown here for *Lasaea cf. nipponica*). Moreover, the relative pro-

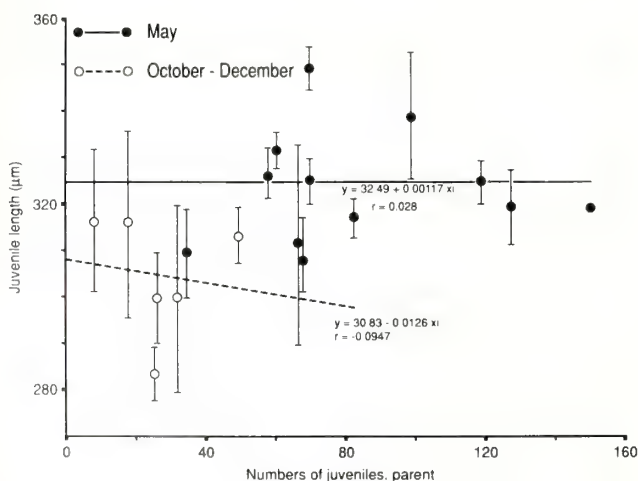


Fig. 5. *Lasaea cf. nipponica*. Juvenile shell length, expressed as a function of the numbers of juveniles/parent, for both spring (May) and autumn (October - December) broods in Hong Kong (mean $\pm$ 1 S.D.).

Table 1. Aspects of the reproductive biology of species of *Lasaea* (after McGrath and Ó Foighil, 1986) compared with those identified for *L. cf. nipponica* from Hong Kong.

Species	<i>Lasaea rubra</i>	<i>L. subviridis</i>	<i>L. rubra hinemoa</i>	<i>L. cf. nipponica</i>
Expression of sexuality	hermaphroditic	hermaphroditic	hermaphroditic	hermaphroditic
Brooding period	summer	year round	year round	two phases (May, October - December)
Brood size (juveniles/parent)	6-22 1-21	2-39 4-30	2-33 4-71	$\bar{x}$ = 24 (autumn) $\bar{x}$ = 81.5 (spring)
Maximum size of brooding adult (mm)	2.4	3.3-3.5	4.1	3.6
Size of released juveniles (μ m)	500-600	530-650 600-700	500-600 510-640	280-340
	Oldfield, 1955; Seed and O'Connor, 1980	Glynn, 1965; McGrath and Ó Foighil, 1986	Booth, 1979; Beauchamp, 1986; Ó Foighil, 1985	this study

portions of developmental stages within the broods of *T. con-fusa*, varied seasonally, as is possibly the case with *Lasaea cf. nipponica* in Hong Kong, suggesting variation due to environmental factors.

In Hong Kong, many subtropical species of bivalves show two phases of recruitment, in spring and autumn, e.g. the freshwater species *Limnoperna fortunei*, *Corbicula fluminea* and *Musculium lacustre* (Morton, 1987) as well as the estuarine species, *Brachidontes variabilis*, *Saccostrea cucullata* and *Gafrarium pectinatum* (Morton, 1988, 1990a,b). A similar pattern is emerging for marine intertidal species, e.g. *Septifer virgatus* (Morton, unpub. data). It is thought possible that an otherwise single peak of reproduction is divided into two, early and late, phases by either high mid-summer temperatures or low salinities as a result of the impact upon Hong Kong of the tropical south-east Monsoon (Morton, 1991). In the case of the freshwater species, summer rain-induced flooding can flush away gametes or newly-released juveniles. Such a suggestion needs verification and is being investigated. If it is true, however, then the local species of *Lasaea*, i.e. *Lasaea cf. nipponica*, could be compared with *L. rubra* in terms of a summer peak in reproduction being divided into early and late phases by environmental extremes. Sea water temperatures in Hong Kong in May and from October - December are, however, far higher than these reported to be optimal for reproduction in both *L. rubra* (~ 7°C - 14.5°C) (Seed and O'Connor, 1980; McGrath and Ó Foighil, 1986), to which it has been compared (Morton and Scott, 1989), and *L. subviridis* (8 - 15°C) (Ó Foighil, 1985) and range between 26°C (May) and 28°C declining to 19°C between October and December. Salinity, pH and dissolved oxygen levels are less variable during the brooding months than at other times of the year (Table 2).

Similarly, with regard to the numbers of brooded

juveniles ($\bar{x}$ = 24 - 81.5/parent) and their maximum size (280-340 μ m), the local species is different from all other species of *Lasaea* for which information is available (Table 1). In comparison with all other species, *Lasaea cf. nipponica* in Hong Kong broods, approximately, twice the number of half the size juveniles.

On the basis of earlier described taxonomic evidence and the data set out above, it is, therefore, concluded that the local species of *Lasaea* is best designated the name, *Lasaea cf. nipponica*.

Ó Foighil (1986) has shown that *Lasaea subviridis* can brood juveniles to either the Prodissoconch I or Prodissoconch II stage (Carriker and Palmer, 1979; Waller, 1981), the former lacking and the latter possessing commarginal striae. Ó Foighil suggests that prodissoconch morphology is environmentally regulated such that the former morphology is produced by submerged adults, the latter by intertidal parents. This study similarly suggests that for *Lasaea cf. nipponica*, juvenile size is different for spring and autumn

Table 2. The ranges of various sea water parameters at Cape d'Aguilar, Hong Kong, and those prevailing during the periods (May and October - December) when *Lasaea cf. nipponica* is brooding juveniles.

	Range	May	October - December
Temperature (°C)	17-30	26	28- > 19
Salinity (‰)	26-33	32	31-32
pH	7.7-8.4	8.1	8.3-8.1
Dissolved oxygen (mg/l)	5.6-7.8	6.8	6.0-6.9
Most probable wave heights (m) (50% exceedance) (After Apps and Chen, 1973)		2.0	2.9

clutches. This observation, however, due to the great variation in the sizes of juveniles comprising, particularly, the spring broods, requires further verification and such data need to be thus treated with caution. In Hong Kong, however, wave heights are higher in autumn than in spring (Apps and Chen, 1973) (Table 2) although the pattern of tidal fluctuations is similar at these times of the year (Morton and Morton, 1983). Autumn parents are thus more frequently immersed (by higher waves) than spring parents. It is thus possible that brood size (and possibly juvenile size) is related to immersion period which, if true, is similar to the situation reported upon by Ó Foighil (1986) for *L. subviridis*. Russell and Huelsenbeck (1989) similarly show for *Transenella confusa* in California that brood size is smaller in winter than at other times of the year.

Brooded juveniles of *Lasaea* do not possess feeding structures (Oldfield, 1964; Beauchamp, 1986; Ó Foighil, 1986). It is, therefore, likely, since the parental size range is the same in spring and winter, that differential environmental factors act upon the *Lasaea* cf. *nipponica* parents in spring and summer to limit brood size in winter. This could be because of seasonal differences in percentage time immersed, acting via seasonal variations in wave height (Table 1). It is possible that such factors act upon the physiological environment of the parental mantle cavity, as suggested for *Ostrea edulis* by Waller (1981) and *L. subviridis* by Ó Foighil (1986).

The results of this study upon *Lasaea* cf. *nipponica* and that of Russell and Huelsenbeck (1989) upon the burrowing *Transenella confusa* suggest that brood size is environmentally regulated. So, apparently, is prodissoconch morphology in *L. subviridis*. Apart from the obvious implications of the former observations with regard to subsequent recruitment and thus population dynamics, such studies collectively suggest that the wide variation in shell form typical of species of *Lasaea* is phenotypic, accounting for the many taxonomic problems surrounding the representatives of this genus.

ACKNOWLEDGMENTS

I am grateful to Mr. T. K. Cheung of the Electron Microscope Unit of The University of Hong Kong for SEM assistance.

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Date of manuscript acceptance: 3 December 1991

Reproductive ecology of the Antarctic bivalve *Lissarca notorcadensis* (Philobryidae)

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Abstract. The reproductive ecology of museum deposited specimens of the small philobryid Antarctic bivalve *Lissarca notorcadensis* was examined. Common to southern polar waters, the philobryids are typically epifaunal in habitat. *L. notorcadensis*, collected from sites adjacent to the Antarctic Peninsula, are found frequently attached byssally to cidaroid echinoid spines. While 95% of all mature clams display either ova or sperm, there is a quantitative shift in populations examined from male to female dominance with some indications of proterandry. *L. notorcadensis* are synchronous pallial brooders producing 9-15 shelled offspring regardless of adult female size. Juveniles that have already produced dissoconch shell, apparently crawl from the parental infrabranchial chamber directly onto the same spine as their parent. The newly released juveniles lack fully developed digestive diverticula but are sustained by residual yolk reserves. Possible adaptations for brooding in the adult include an absence of the anterior adductor muscle thereby increasing pallial volume, and filibranch ctenidia that allow compact brood development. We suspect that these iteroparous bivalves release their broods in late austral winter to early summer. A single distinct collection from a site close to South Georgia reflects differences between zoogeographic areas.

As environmental threats to the relatively pristine ecosystem of Antarctica grow, it is imperative that we examine its present malacofauna and ecological "sanctity". The philobryid bivalves are a common and widely distributed group of southern polar molluscs (Powell, 1960; Tevesz, 1977) with intriguing reproductive biologies. Primarily thought to be dioecious, the philobryids brood their young in their infrabranchial chamber well past prodissoconch stages (Prezant, 1990). The circumantarctic philobryid *Lissarca notorcadensis* Melvill and Standen has been reported to have a small prodissoconch I and a large prodissoconch II (Prezant, 1990), a pattern more characteristic of bivalves producing planktotrophic larvae (Jablonski and Lutz, 1980; 1983). The proposed discrepancy in larval shell form could be a retention of a primitive character from a nonbrooding ancestry. Philobryids, while Tertiary in origin (Morton, 1978), have in fact an interesting mix of apomorphic and plesiomorphic characters (outlined in Tevesz, 1977; Morton, 1978).

The Philobryidae are known poorly. Tevesz (1977) defines the family as filibranch bivalves with a posteriorly directed, ventral ciliary tract and a ligament pit situated between two rows of denticles (the provinculum). According to Tevesz (1977) there are eight valid, extant genera, distinguishable by variations in their ligament pit, denticles, adult hinge teeth, external shell ornamentation, and adductor muscles. The family is also characterized by nonfused mantle lobes, lack of siphons, reduction or absence of anterior adductor muscle, and the capacity to brood young to a juvenile stage. There has, to date, been only a single detailed report

on the reproductive ecology of any philobryid. Richardson (1979) reported on the reproductive ecology of a shallow water population of *Lissarca miliaris* (Philippi) from Borge Bay, Signy Island, Antarctica. Other than this, Dell (1964), reported about 400 "young shells" associated with the ctenidia of the Antarctic philobryid *Philobrya capillata* Dell. An incidental report on philobryid reproduction is given in Bernard (1897). *L. notorcadensis*, while sometimes found as an epibiont on kelp holdfasts, gorgonians, hydroids, sponges, and large solitary ascidians, is frequently found byssally attached to cidaroid echinoid species [especially species of the genus *Ctenocidaris* (J. H. Dearborn, pers. comm.)] (Fig. 1). The present paper details some aspects of the reproductive biology of *L. notorcadensis* from deeper Antarctic waters.

Viviparity in antarctic fauna appears to be a common phenomenon but understanding of this stems from studies of relatively few species (see Pearse *et al.*, 1991). In the very early 1900s, Giard (1905) suggested that antarctic organisms would tend to have a disproportionately large number of brooders that bypassed larval stages. Thorson (1950) proposed that the large number of polar brooders could be attributed to the short austral summers and restricted food supplies. Undoubtedly, the philobryids are well adapted to brooding but this may not be an adaptation to polar conditions but instead could be a conservative trait reflective of the group's phylogeny. Possible adaptations of adult female philobryids for brooding young to late stage juveniles include the absence of an anterior adductor muscle, filibranch ctenidia with a reduced outer demibranch, and a one way flow of pallial water

currents originating along the anterior portion of the mantle cavity. The relationship between small adult size and internal brooding is discussed by Strathmann and Strathmann (1982). This paper has a more narrow focus and attempts to explore the overall reproductive ecology of a single, small antarctic

species with respect to brood size, maturation characteristics, and distribution within habitat.

METHODS

Specimens of *Lissarca notorcadensis* were obtained from the Smithsonian Oceanographic Sorting Center of the U. S. National Museum. Voucher specimens have been accessed into the Smithsonian Institution, U. S. National Museum of Natural History (USNM Cat. #860303). Specimens were collected during expeditions of the early to mid-1970s and included the following: Islas Orcadas Cruise 575 from 12 May 1975 using a 10' Blake Trawl at depths of 132 - 143 m from 53°38.0'S/038°01.8'W; 2 June 1975 using a 5' Blake Trawl at depths of 161 - 210 m from 56°23.8'S/027°24.6'W; and 31 May 1975 using a 5' Blake Trawl at depths of 121 - 228 m from 56°42.8'S/026°09.7'W; and the HERO Cruise of 3 January 1972 using a Blake Trawl at a depth of 110 m from 64°47.3'S/64°07.4 to 64°06.3'W. Locations are denoted on a station map (Fig. 2). More specific information on the Islas Orcadas cruise can be found in DeWitt (1976). Most of these cruises were under the supervision of Dr. John H. Dearborn, University of Maine. While only spine material was available to us and positive identification of the specific host proved difficult, it is most likely that the host echinoid was one of three common cidaroids present at these collection sites: *Ctenocidarid geliberti*, *C. perrieri* or *C. speciosa* (J. Dearborn, pers. comm.). Specimens were preserved originally in 10% formalin and transferred to isopropyl

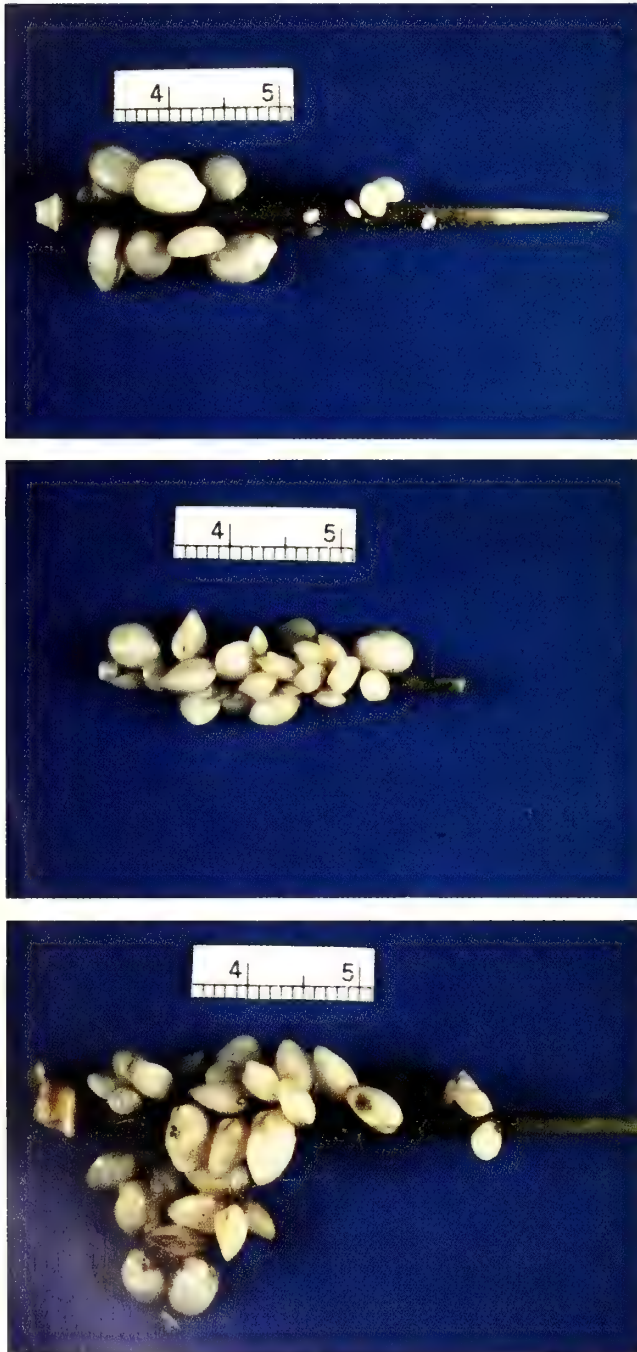


Fig. 1. *Lissarca notorcadensis* attached to sea urchin spines. Various distributions and concentrations are displayed by these three "typical" examples of spines recovered.

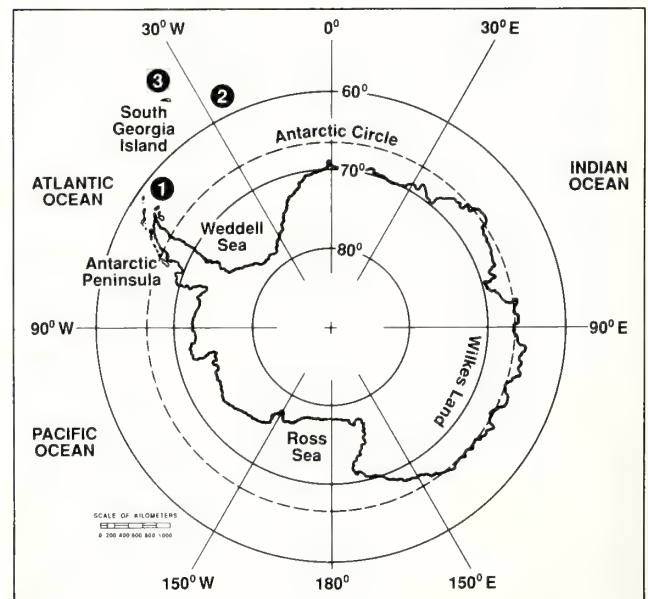


Fig. 2. Antarctic station map. Exact locations are noted in the text. Site 1, 3 January 1972 collection; Site 2, 31 May and 2 June 1975 collections; Site 3, 12 May 1975 collection.

alcohol within two weeks of collection (B. Burch, pers. comm.). They were transferred to 70% ethanol in February 1986.

To obtain specific data on sex ratios, stages of development, and reproductive and brood capacity, clams from the 3 January 1972, 31 May and 2 June 1975 samples were divided into six size classes based upon maximum height (<1.0mm, 1.0-1.99mm, 2.00-2.99mm, 3.00-3.99mm, 4.00-4.99mm, and >5.00mm). A further breakdown of size classes at the 0.5mm level was also instituted in order to resolve more finely the possibility of an ontogenetic shift in sex ratio. Fifty specimens from each size class had their length, height and breadth measured to the nearest 0.1mm. Each specimen was then opened and examined under a dissecting scope to determine sex, number of mature ova, number of nonshelled embryos, and number of shelled juveniles in brood. Maturity of ova was determined by size, mature ova being much larger than immature. Histological subsamples were taken to confirm maturity of ova. There was a clear distinction between shelled and nonshelled embryos with, in our samples, very few intermediates.

To determine if there was any segregation of stages or sex along individual echinoid spines, 15 spines from 12 May 1975, and 34 spines from 31 May 1975 were photographed carefully from two angles to expose all attached clams. These two collections were used because of their sampling time proximity. Only spines containing five or more byssally attached clams were examined. Each individual clam was numbered and the numbers keyed into similarly numbered photographs. Each numbered clam was then removed from the spine, measured to the nearest 0.01 mm and examined for sex, mature ova, nonshelled and shelled brood. Each clam was placed into one of three spine sectors determined in two ways. First, the entire spine from base to tip was divided into thirds. The distance of each specimen was measured from the base of the spine to the point of byssal attachment on the spine. These clams were then placed in categories of top, middle or bottom third of a total spine. To account for segregation *within* a population on a spine, clams were also measured in terms of their position within a particular spine population. Thus, the lowest attached clam represents the position of the lowest measurement; the highest clam on a spine represents the top measurement. With these two demarcations, the individual spine populations were again divided into thirds and clams were denoted as being in one of the three intrapopulation sectors.

Statistical evidence for our conclusions are given along with the collation of data in the results section. All statistical decisions were made at the $\alpha = 0.05$ level, however the actual probabilities in each case are also given. One way analyses of variance (ANOVA) were conducted to test for possible differences among the four specified groupings (i.e. male, female with ova, female with nonshelled embryo, female with shelled

juveniles) with respect to length, height and breadth of the clams. Tukey's Test was also conducted as a follow-up specific comparison test to determine pairwise differences among the four groups. Strength of Association measures were also calculated. One-way ANOVAs were also conducted to test for differences among sample dates with respect to the number of nonshelled embryos and number of shelled juveniles in brooding females. Since the data consisted of counts, square-root transformations were used.

Combining the data from all spines examined, chi-square tests were done to compare the groupings within populations along the bottom, middle, and top thirds of the *entire* spine and also the populations demarcated by the uppermost and lowest clam (i.e. *within* spines). Pairwise specific comparisons were conducted when the initial analysis showed significance. Further chi-square tests were performed to examine the relative abundance of each grouping within a segment of the spine.

Chi-square tests and pair-wise specific comparisons were also used to determine if different *patterns* existed between any specific grouping with respect to the distribution of clams along the bottom, middle and top third of the entire spine (and also within a population on a spine).

RESULTS

Sexes typically appear separate in *Lissarca notorcadensis* although a small percent (less than 5%) of the population examined, especially in the 3.5 - 4.0 mm size range, possess both testes and ovaries. Another indication of protandric hermaphroditism includes proportionally more males than females of smaller size. A compilation of total number of males versus females in the combined 31 May 1975 and 2 June 1975 samples reveals that approximately 73% of the mature population for the size class between 3.00 and 3.99 mm were males ($n = 159$); between 4.00 and 4.50 mm 55% were males ($n = 166$); between 4.51 and 4.99 mm 35% were males ($n = 101$); and over 5.00 mm 39% were males ($n = 31$) (Fig. 3). These numbers do not change substantially when specimens from 3 January 1972 are incorporated into the calculations (respectively males then compose 66%, 54%, 33%, no specimens in the largest size range were collected on this date). Thus there is an inversion from approximately 70% of the total population being male at sizes under 4.00 mm to about the same percent being female at sizes above 4.50 mm. About equal numbers are diagnosed as male or female in the 4.00 - 4.50 mm range. Recall it is also in this size range where we find most specimens possessing both gamete types.

An examination of mean lengths, heights and breadths for each grouping reveals a clear trend (Table 1). For all three size variables measured, the males are the smallest, the

Protandry

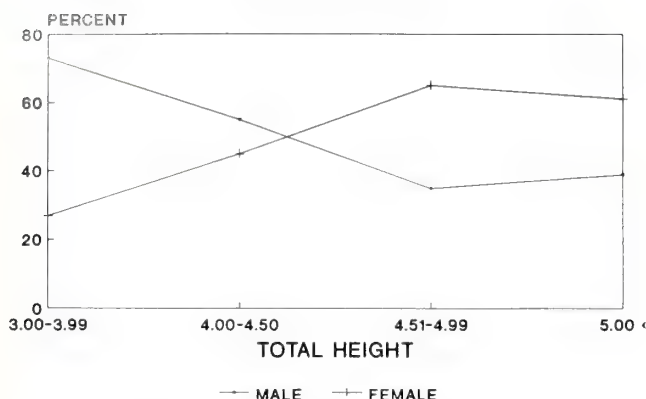


Fig. 3. Total percent male versus female in incremental size classes. The graph represents cumulative totals in each size class from 31 May and 2 June 1975. The cross-over point also represents the approximate size class that has shown the greatest number of simultaneous hermaphrodites.

females with ova are next to the smallest, brooding females with nonshelled embryos are next to the largest and brooding females with shelled juveniles are the largest. Statistically, for all three variables, males were significantly smaller than both brooding female groups, while females with ova were significantly smaller than females with shelled juveniles. Although males were not significantly smaller than females with ova and females with ova were not significantly smaller than females with embryos, the trend remains intact. As would be expected, the correlation between the three measurement variables is strong. For example, the correlation coefficient in a sample of males ($n = 110$) is 0.95 for length and height.

Strength of Association measures for length, height, and breadth respectively are 0.105, 0.125 and 0.113. This means, for example, that 10.5% of the variation observed in the length data can be accounted for by differences in the specific groupings identified, whereas 89.5% of the variation is due to the combination of the influence of other factors (e.g. environment) on length not accounted for in this analysis.

A mature female *Lissarca notorcadensis* generates relatively few (approximately 25-35) large eggs packed with

yolk. Mature eggs average 140 μm in diameter. There is little variation in number of ova produced with adult growth as most clams examined containing mature ova, contained the same approximate number. Eggs are most likely fertilized in the pallial cavity immediately after release through the paired oviducts.

Lissarca notorcadensis broods its young in the infrabranchial chamber, showing several adaptations that could enhance brooding capabilities. The filibranch ctenidium, with firm but few ciliary junctions, allows spacious volume for development of a brood of few but large embryos and juveniles. Additionally the absence of an anterior adductor produces a spacious pallial cavity for the developing brood. We suspect that the pallial current, flowing from anterior to posterior, also support brood maintenance. There are no indications of any material tissue connections to the developing brood. A preshelled embryo and shelled juvenile are the most common brood stages found in our samples (although very rarely we found partially shelled embryos with merely incipient shell formation). The two stages commonly found in our samples are found independently and do not overlap in a single brood. Both stages contain large quantities of yolk droplets. In the shelled juvenile these yolk droplets surround the developing digestive diverticula.

TEMPORAL ASPECTS. Males form about 60% of the total population of specimens collected on 31 May and 2 June 1975 but only 40% on 3 January 1972 in geographically closely situated samplings. The sample from near South Georgia had 45% males on 12 May 1975 (Fig. 4).

Taking into account the disparate years of sampling, there appears to be a decline in females brooding nonshelled young from January to June. Numbers are quite low for total females with shelled brood in January 1972 but reach a higher plateau in the May-June 1975 samples (Fig. 4). Females with mature ova in January 1972 and 12 May 1975 compose 40% of the total female population rising to over 60% in late May and early June 1975 (Fig. 4). Over 50% of the total female population in January 1972 were collected with early broods. Only 22% of the females on 2 June 1975 contained early broods. In all these data there is the problem of temporal gaps and various sampling sites that can confound the results. Nevertheless, the trends appear in our limited sample set.

DISTRIBUTION ON SPINES

Size Distribution. Size class distributions of specimens from 12 and 31 May 1975 and 2 January 1972, still attached to spines are displayed on the percentage polygon in figure 5. Clams between 2.1 - 4.0 mm in height dominate collections from 12 May 1975 ($N = 208$). The collection from 31 May 1975 ($N = 414$) shows a slight bimodal distribution with peaks in size classes between 1.6 - 3.0 and 3.6 - 5.0 mm. The

Table 1. Average ($\pm$ S.D.) lengths, heights, and breadths (in mm) of the four groupings in cumulative samples of *Lissarca notorcadensis* ($n = 195$) from May, June 1975 and January 1972.

Grouping	Length	Height	Breadth
Males	3.98 $\pm$.57	4.75 $\pm$.89	2.64 $\pm$.56
Females with ova	4.14 $\pm$.50	5.00 $\pm$.87	2.81 $\pm$.53
Females with embryos	4.43 $\pm$.36	5.56 $\pm$.60	3.09 $\pm$.35
Females with shelled juveniles	4.59 $\pm$.40	5.81 $\pm$.64	3.27 $\pm$.39

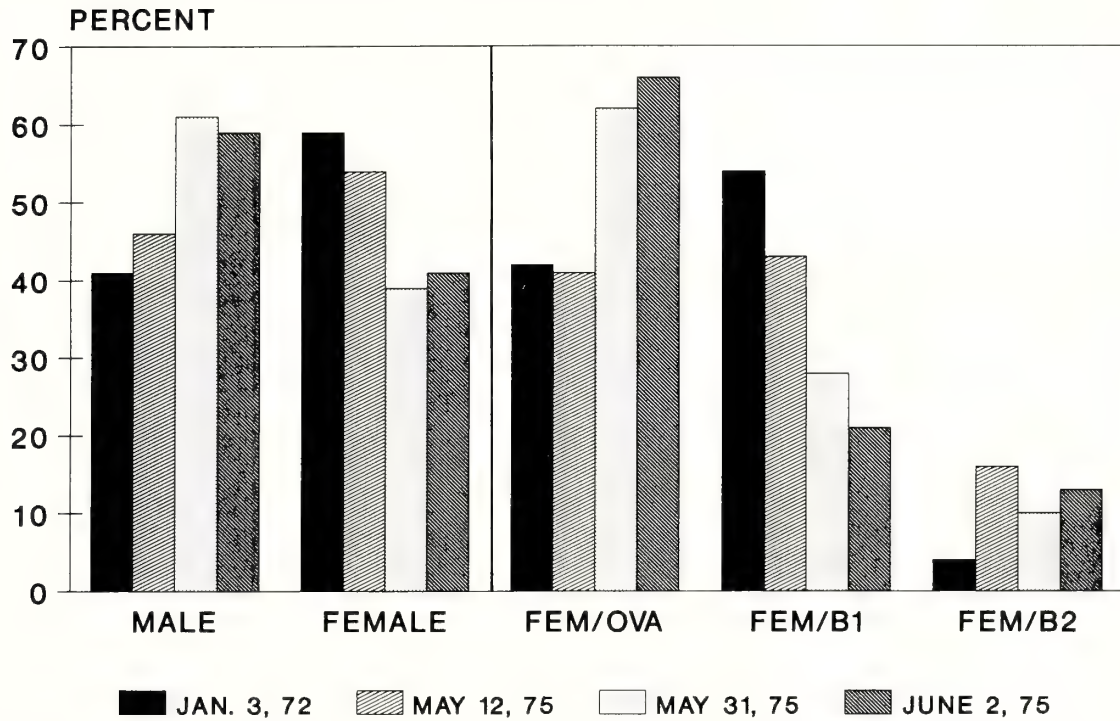


Fig. 4. Proportion of male versus female clams from the four collection dates. The two groups of bars to the left of the vertical line compare total male and female. To the right of the vertical line, the bars represent the break-down of stages of females only. Thus, for 3 January 1972, about 42% of all females had mature ova. FEM/OVA = females with mature ova; FEM/B1 = females with nonshelled brood; FEM/B2 = females with shelled brood. 3 January 1972, N = 85; 12 May 1975, N = 348; 31 May 1975, N = 575; 2 June 1975, N = 187.

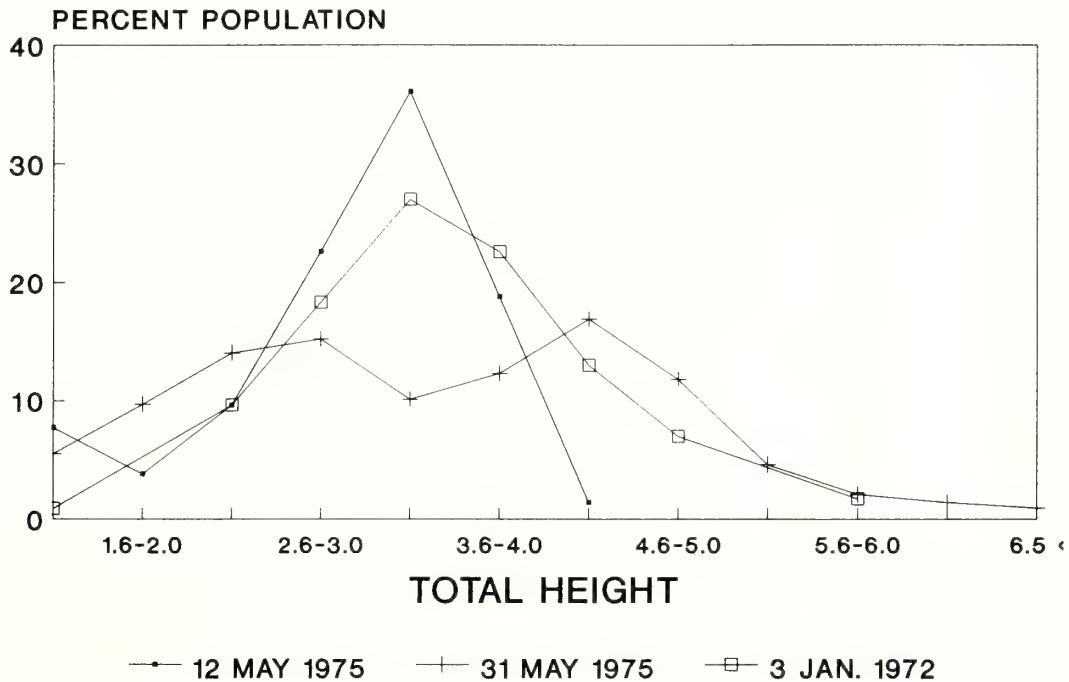


Fig. 5. Length percentage polygon for populations occurring on spines only at three collection dates. The x-axis represents total height in mm; y-axis percent of total population from specific collection date.

size distribution of the January 1972 collection ($N = 115$) is similar to that of 12 May 1975 in that 77.5% of the 12 May 1975 population examined falls between 2.6 and 4.0 mm length and 67.9% of the January 1972 collection falls into the same size range. Only 37.6% of the 31 May 1975 population falls into this size range and it is only this population, as well, that has adults forming a substantial component above the 5.1 mm length.

Sex and Stage Distribution. For the 49 spines analyzed (Fig. 6), a trend for the *entire* spine distribution of decreasing numbers of clams from bottom to top third of the spines was detected for all groupings. Indeed, except for immature clams, more clams are found on the bottom third of the spines than the upper two thirds combined. In general, few clams were found on the upper third of the spines. Figure 7 diagrammatically shows the distribution and significance of all groupings analyzed along the entire spine. A similar representation is seen in figure 8 for *within* spine population distribution.

Even though all groupings exhibited the same general trend of decreasing numbers from bottom to top third, the data were examined further for possible differences between groupings in the relative "steepness" of the overall decreas-

ing pattern. No significant difference was found in the distribution pattern of clams among the bottom, middle and top third of the entire spine between males and total females ($p > .18$), males and females with ova ($p > .87$), females with nonshelled embryos and females with shelled juveniles ($p > .93$), total females and immature clams ($p > .05$), females with embryos and immature clams ($p > .50$), females with shelled juveniles and immature clams ($p > .66$), females with ova and all brooding females ($p > .23$) and all brooding females and immature clams ($p > .39$). A significant difference, however, in the pattern was found between males and all brooding females ($p < .04$), males and immature clams ($p < .001$) and females with ova and immature clams ($p < .03$). A significantly greater proportion of males than brooding females was found on the bottom third compared to the top third of the spines ($p < .01$) and also on the middle third when compared to the top third ($p < .05$). No difference in distribution pattern was significant for these two groupings when comparing the middle with the bottom third of the spines ($p > .49$). A significantly greater proportion of males than immatures was found on the bottom third compared to the middle ($p < .004$), on the bottom compared to the top ($p < .001$) and on the middle compared to the top ($p < .03$). A significantly greater proportion of females with

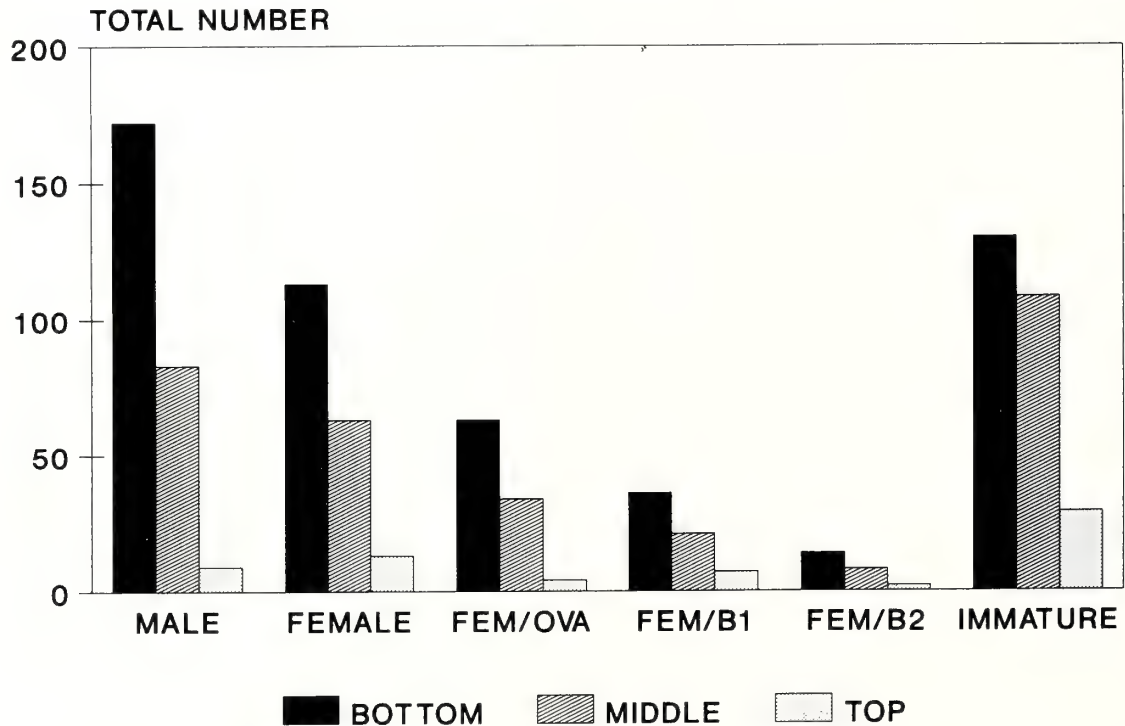


Fig. 6. The distribution of clams along total spine sectors. Bottom = bottom third of total spine; Middle = middle third of total spine; Top = top third of total spine. y-axis is total number of clams of specific stage or sex. FEM/OVA = females with mature ova; FEM/B1 = females with nonshelled brood; FEM/B2 = females with shelled brood; Immature = clams, usually less than 3.0 mm in height, with nondifferentiated gametes. 49 spines analyzed from 12 and 31 May 1975.

ova than immatures were found on the bottom third compared to the top ($p < .02$), however the distribution patterns of these two groupings were not significantly different when comparing the bottom with the middle ($p > .08$) and comparing the middle with the top ($p > .13$).

Examining the bottom third of the spines, the region with the greatest concentration of clams, reveals that there is a progressive decline in total number from male to total female ($p < .05$) and, within females, from mature females with ova to females brooding nonshelled embryos ($p < .05$) and shelled juveniles ($p < .05$) (Fig. 6).

Because spines only infrequently have clams attached along their entire length, separate measurements of abundance were taken by dividing the length of spine that did contain clams into thirds. This accounts for what we have termed *within* population distributions. Figure 9 denotes the cumulative distribution of clams on 49 spines for 12 and 31

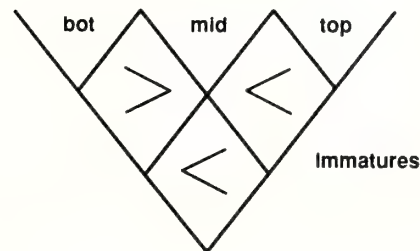
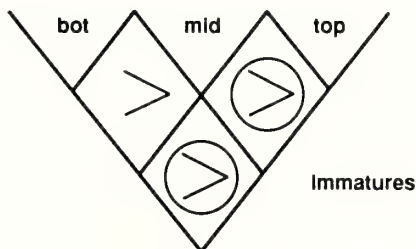
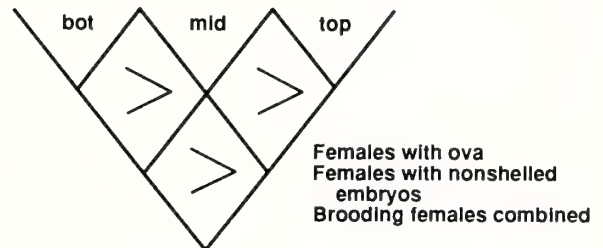
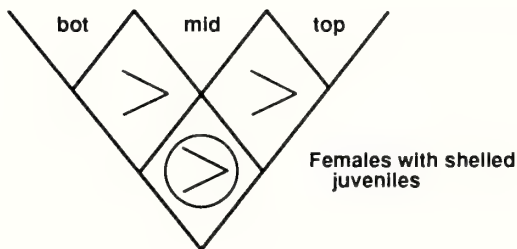
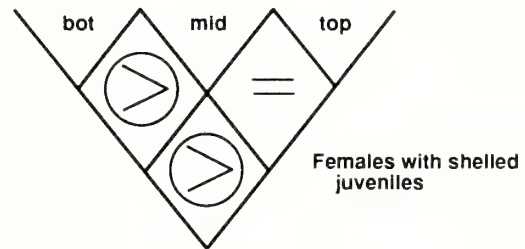
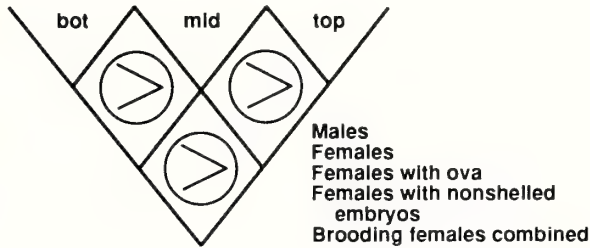
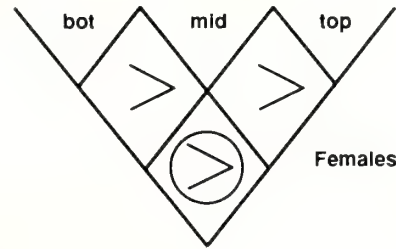
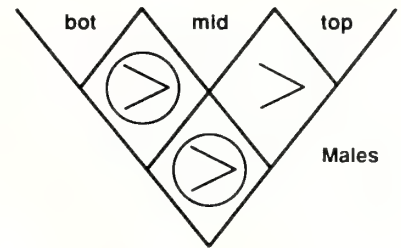


Fig. 7. A diagrammatic representation of clam grouping distributions along entire spine length. Bot = bottom third of spine, Mid = middle third, Top = top third; > = greater than, < = less than, circled symbols are statistically significant.

Fig. 8. A diagrammatic representation of clam groupings distributed *within* a given spine population. Symbols and abbreviations as in figure 7.

May 1975. The trend seen on the *entire* spine of decreasing numbers of clams from bottom to top is not exactly seen in the *within* spine populations (compare Figs. 7 and 8). Some of the groupings were more evenly distributed within the spine. Figure 10 shows distribution of each grouping on the spines compared against themselves, with the exception of juveniles, again demonstrating the reduction in numbers with ascent on the spine.

Possible differences between groupings in distribution patterns of clams among the bottom, middle and top thirds within the spine population were also checked. No significant differences were found in the distribution patterns between males and females ($p > .34$), females with ova and all brooding females ($p > .64$), males and all brooding females ($p > .84$), females with nonshelled embryos and females with shelled juveniles ($p > .24$), males and females with ova ($p > .23$), females with ova and immature clams ($p > .15$) and females with embryos and immatures ($p > .16$). A significant difference in pattern was found between males and immatures ($p < .05$), brooding females and immatures ($p < .05$) and females with shelled juveniles and immatures ($p < .05$). Except for the comparison between males and immatures, all of these significant pairings are different than those found to be significant for the *entire* spine. Similar to the earlier test for the entire spine, a significantly greater proportion of males than immatures was found in the

bottom third compared to the top third ($p < .001$) however no statistical difference was exhibited between the middle and top ($p > .09$). A greater proportion of females than immatures was found in the bottom third compared to the top ($p < .01$) and more in the middle than the top ($p < .05$). No significant difference was observed between the bottom and middle ($p > .45$). A greater proportion of all brooding females than immatures was observed in the bottom third of the population than the top ($p < .01$) but no difference in pattern was detected for these two groups between the bottom and middle ($p > .27$) and between the middle and the top ($p > .14$). This same result is exhibited when comparing females with juveniles and immatures. Since females with shelled juveniles are a part of the grouping of all brooding females, the previous result could be due to the overriding influence of the females with juveniles in this grouping. In particular, a greater proportion of females with juveniles than immatures was observed in the bottom third than the top ($p < .05$), however no differences were detected between the bottom and middle ($p > .05$) and middle and top ($p > .77$).

Brood Distribution. Table 2 shows the average number of nonshelled embryos and shelled juveniles found in broods from each collection. The average number of nonshelled embryos within a brooding female was significantly higher in the 31 May 1975 sample (avg. = 14.4) than in either

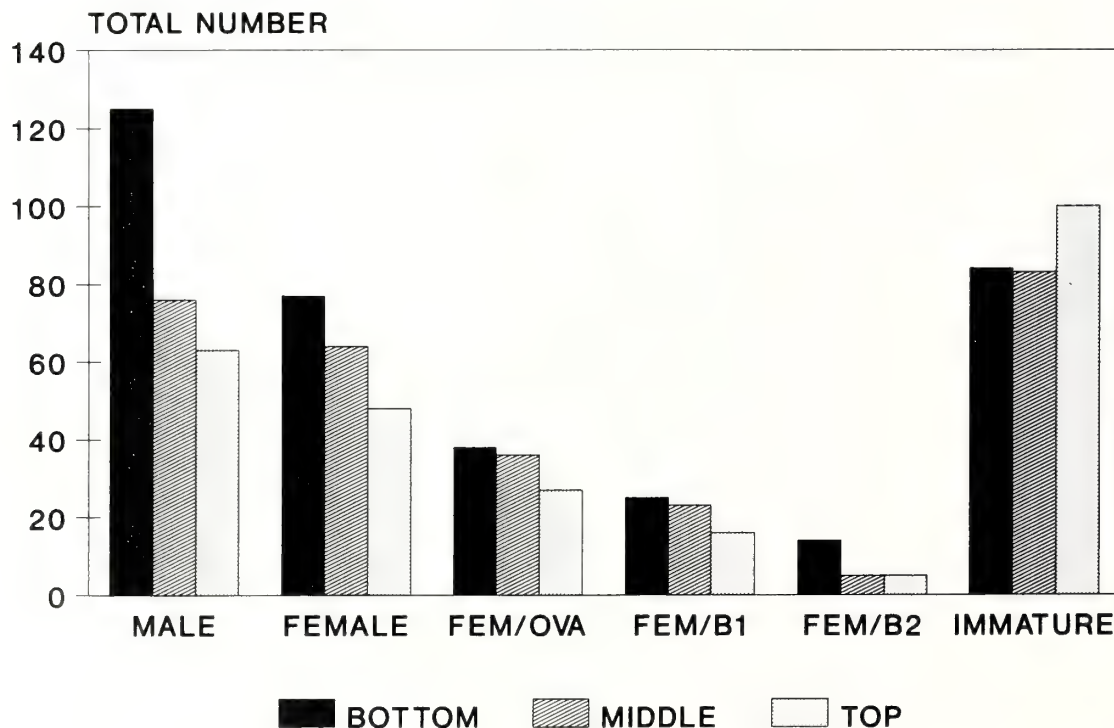


Fig. 9. Total number of clams in each sector from each sex and stage within population on echinoid spines. 49 spines analyzed from 12 and 31 May 1975.

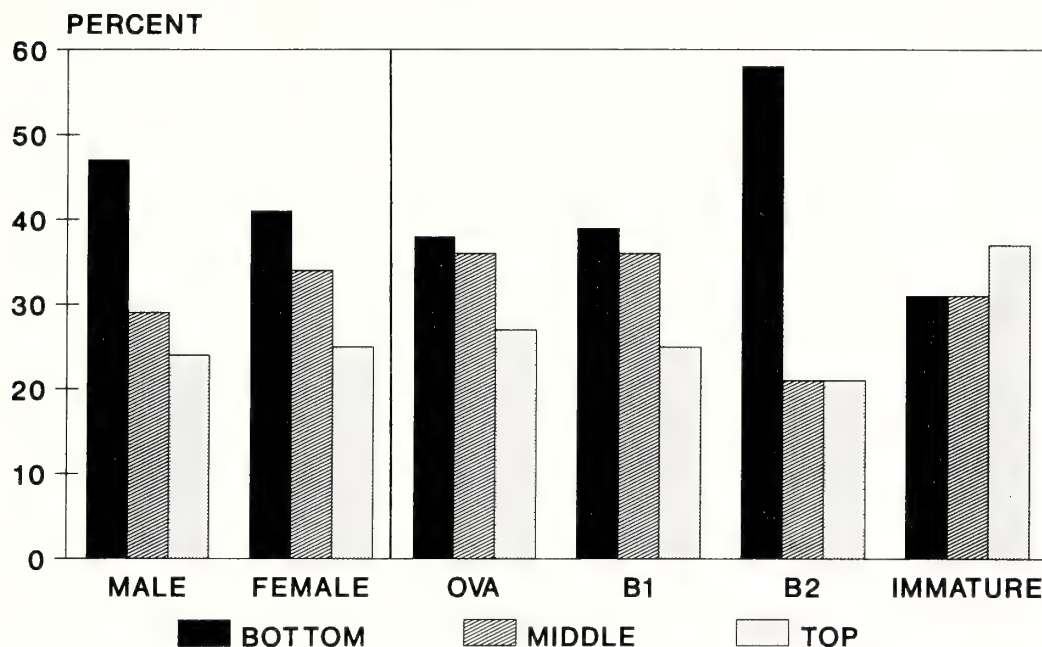


Fig. 10. Percent of total sex and stage in each sector of echinoid spine. Bars for females with ova, nonshelled and shelled brood, represent percent of total female population, not total population. Population as per figure 6.

the 2 June 1975 (avg. = 10.9, $p < .05$) or 12 May 1975 (avg. = 9.5, $p < .05$) samples. Furthermore, the 3 January 1972 sample (avg. = 12.9) is significantly higher than the 12 May 1975 sample. There are no statistically significant differences between the 31 May 1975 and 3 January 1972 samples, or between the 2 June 1975 and 3 January 1973 samples, or between the 2 June 1975 and 12 May 1975 samples.

In contrast, when comparing the number of shelled juveniles within a brooding female among the sample dates, no significant differences were found ($p > .05$). The 3 January 1972 sample was not included in the analysis since it contained only a single adult brooding shelled juvenile.

PROPOSED LIFE CYCLES

Based on samples from 3 January 1972, 31 May and 3 June 1975, the following life cycle is proposed for *Lissarca notorcadensis* (see Fig. 11). Fertilization occurs in the mantle cavity after release of mature eggs in the early austral summer or late spring. During the summer, when females dominate in total numbers over males, many females are brooding early stage, nonshelled embryos. Shell development begins during the late summer and early autumn with simultaneous redevelopment of mature ova. Many females brooding late stage young also possess mature ova. In late autumn and early winter, males dominate females in abundance and ova are fully developed. Among females, those with mature ova dominate at a time when males dominate the total population. Shelled juveniles are released in late winter to early summer. Only a single adult was found

brooding shelled juveniles in the January 1972 sample.

This basic cyclic trend (Fig. 11) is superimposed on populations where each stage can be found at some level in the population year round and is characteristic for stations sampled in January 1972, and 31 May and 2 June 1975. That for the 12 May 1975 sample is slightly offset, possibly reflecting the proximity to South Georgia. It is possible that the conversion of some portion of the population from males to females also occurs at a specific season, perhaps just after austral summer, accounting for some of the differences in sex and stage noted. Some of the basic features of this life cycle are noted in Table 3 along with a comparison of the same features for *L. miliaris*.

DISCUSSION

The Antarctic Ocean benthos, below the 33 meter limit of anchor ice formation, offers a rich and diverse assemblage including close to 900 species of molluscs (Moss, 1988). Most of our knowledge of the benthos reflects research that has occurred over the past three decades. Many collected materials have been deposited in various museums and much remains to be discerned from these stored collections that essentially remain untouched.

The Smithsonian Institution Oceanographic Sorting Center represents a rich repository of historical collections of Antarctic molluscs. Many of these collections were obtained during expeditions of the early to mid-1970s. The present research examined specimens available from those

Table 2. Average number ($\pm$ S.D.) of nonshelled embryos and shelled juveniles retained in brood of females from various sampling times and sites. The collection for 12 May 1975 is adjacent to South Georgia Island. The other collections are closer to the Antarctic Peninsula. Note that the 3 January 1972 sample had only a single female brooding shelled juvenile.

Date	N	Average number of nonshelled embryos	N	Average number of shelled juveniles
3 Jan 1972	24	12.9 $\pm$ 4.1	1	10.0
12 May 1975	32	9.5 $\pm$ 2.7	15	8.9 $\pm$ 2.5
31 May 1975	35	15.1 $\pm$ 4.2	27	10.4 $\pm$ 4.9
2 June 1975	17	10.9 $\pm$ 3.2	10	9.0 $\pm$ 3.1

collections and as such was limited to samples outlined in the methodology. Before discussing the results per se, we must ask, can we lend confidence to interpretations based on a rather disparate set of Antarctic collections? The tentative answer is yes, at least until we have a more complete set of data with more closely set samples over a much longer period of time and from more defined benthic sites. Additionally, the clear clues offered in the analyzed data set in conjunction with data from the work of Richardson (1979) on *Lissarca miliaris*, lends additional credence to our speculation. *L. notorcadensis* offers a classic example of a polar brooder leaning heavily towards the "k-selection" side of an r-K continuum [as designed by Pianka (1970)]. K-selected species vie for maximum competitive ability in a "saturated" environment (as outlined by Southwood, 1977) with slow growth, delayed maturation, iteroparity, low fecundity and large yolky eggs. It is recognized, however, that an examination of living populations through time is called for to refine our concept of reproductive ecology of this philobryid.

Berkman *et al.* (1991) examined recently the planktonic development of the Antarctic scallop *Adamussium colbecki* (Smith) and suggest that various anatomical features prohibit brooding. Included in these restraints are a filibranch gill without "strong tissue connections", an open mantle cavity without mantle edge connections, and a lack of siphons. Considering these same characters in *Lissarca notorcadensis*, it is doubtful that these inhibit internal pallial brooding. *L. notorcadensis* brood their young in their relatively spacious infrabranchial chamber. Increased volume for brooding results from a lack of anterior adductor muscle and a reduced outer ctenidial demibranch. We suggest that flexibility of the filibranch ctenidial filaments allows the growing brood to essentially impinge upon the gills without disruption of function. The ctenidial filaments are relatively short but "stout". Burne (1920; p. 238), in fact, suggests that the gills "present a somewhat embryonic appearance." The anterior flow of pallial water across the gills, as discerned for other philobryids by Tevesz (1977) and Morton (1978), can serve to efficiently aerate the developing young, which presumably

are retained in brood for an extended period of time.

Large yolky eggs, as found in *Lissarca notorcadensis*, are typical of direct developing bivalves (Matveva, 1978). Similarly large eggs are found in other brooding philobryids (eg. *L. miliaris*; Richardson, 1979) as well as several other brooding bivalves including species of *Lasaea* Beauchamp, 1986; McGrath and Ó Foighil, 1986), *Transennella tantilla* (Gould) (Asson-Batres, 1988) and *Kellia suborbicularis* (Montague). Thorson (1950) suggested that eggs of many polar marine invertebrates are large and have dense concentrations of yolk. This suggests that reproduction in polar habitats is expensive energetically and would require large energy stores (Clarke, 1979). Vance (1973a, b) suggested that it is more efficient to produce fewer but larger eggs in a uniform, stable environment as opposed to a variable or unstable environment. The large and yolky eggs of the philobryids, once fertilized, give rise to slowly developing young that are retained in the pallial cavity past prodissoconch stages and into an early dissoconch stage. When released, the young of *L. notorcadensis* have a full complement of organs including a nearly complete digestive system. They retain, however, a quantity of yolk that surrounds the developing digestive diverticula. It is not certain whether the newly released brood actively feed but it is unlikely. Similar stores of apparent nutrients (yolk) in released young are also found in direct developing *Lasaea* (Ó Foighil, pers. comm.) and in another Antarctic bivalve, *Kidderia subquadratum* Pelseneer (Shabica, 1974). In the latter, Shabica (1974:59) also found that "digestive diverticula of the embryos remain nondifferentiated until approximately two months post-liberation suggesting that no active uptake of particulate nutrients occurs while the embryos are developing within the maternal organism or even immediately after liberation." Additionally Shabica found that "large amounts of yolk" were widely distributed in the early

Table 3. Comparative data for *Lissarca notorcadensis* and *L. miliaris*. Data for *L. miliaris* is adapted from Richardson (1979). Sex size ratio signifies comparative sizes of males and females at various stages. Sex ratio indicates total number of male versus female. ¹See text for clarification.

	<i>L. miliaris</i>	<i>L. notorcadensis</i>
Depth	5-9 m (to 121 m)	100-400 m (to 1000 m)
Maximum height	6.0 mm	7.0 mm
Height at maturity	2.5 mm	2.9 mm
Brooding height	3.1 mm	3.6 mm
Average brood	60	9
Brood range	54-70	6-16
Pre-release size	0.57 mm	1.1 mm
Sex size ratio	1:1	female > male ¹
Preshelled brood	Feb. - July	Summer (Jan.; some year round)
Partial shelled	Aug. - July	
Shelled brood	Nov. - Dec.	By May
Recruitment	Dec. - Feb.	June - Dec. ¹

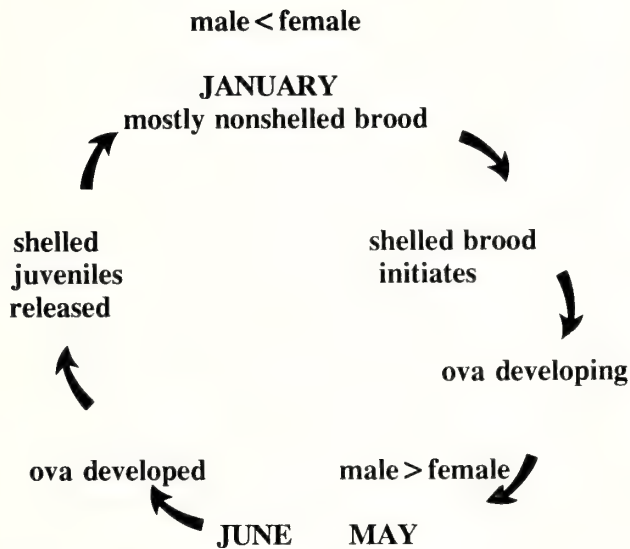


Fig. 11. Proposed life cycle for *Lissarca notorcadensis*.

embryo of *K. subquadratum* but later surrounded developing organs. In fact, as noted by Shabica (1974:iii), yolk was still present in newly released embryos, which can continue development "...at least for short periods, outside the maternal organism." We have predicted that *L. notorcadensis* release shelled brood in late winter to early summer. In cases where the brood is released in later winter, these presumed nutrients may well serve to satisfy nutrient requirements prior to the end of the austral winter and up to a time when primary production increases in the Southern Sea. It is also possible that the final stages of development occur post-release as they do in *K. subquadratum*. On the other hand, Ralph and Everson (1972) speculate that the brooded young of another Antarctic bivalve, *K. bicolor* (Martens), could remain in the parental mantle cavity for "their first winter" prior to release. This is certainly possible for *L. notorcadensis* as well and in fact the dissoconch shell deposited prior to release could indicate this extended brood. There is, however, no way of knowing with certainty until monthly samples are available.

The brood of *Lissarca notorcadensis* develops synchronously, as in the intertidal Galeommatoidean *Lasaea rubra* (McGrath and Ó Foighil, 1986). Prior to brood release, the developing young have all deposited significant dissoconch shell material and young are released as juveniles. Numerous females examined that contained developing broods were also well into a renewed oogenesis cycle (unpub. data). There appears to be a decrease in number of mature ova to early nonshelled brood and finally shelled juveniles. Unlike *L. rubra* (McGrath and Ó Foighil, 1986) and *L. miliaris* (Richardson, 1979), ultimate brood size in *L. notorcadensis* is very small and is independent of adult size. The smallest as well as the largest specimens averaged 9.4 shelled young. Similarly, there was an average of 12.1 nonshelled young in broods of com-

parable sized animals and these in turn were generated from a potential field of at least 25 eggs. The decline is specifically unaccounted for but we assume a loss once eggs are released into the infrabranchial chamber which is essentially open to the environment since the adult mantle edges remain unfused. Decline in the potential number of recruits was noted by Shabica (1974) who discussed "sloughing-off of eggs" in the brooding Antarctic bivalve *Kidderia subquadratum*.

Young of *Lissarca notorcadensis* are released presumably from the pallial brood chamber directly into their parent's microhabitat. At time of release the byssal gland is fully functional and young can attach immediately to either the home spine or the shell of another member of the population. In either case, the young are released into the immediate vicinity of the adult and hence obtain whatever benefits are derived from this microhabitat. An association between bivalve and sea urchin is not unique to this philobryid. *Axinodon symmetros* (Jeggreys) is, for instance, associated with deep water echinoids (Ockelmann, 1965). The symbiosis for *L. notorcadensis* appears to be commensalistic with the bivalve being held above the sediment and in the water column where filtration efficiency could be increased due to lack of sedimentary interference. Additionally, the bivalves obtain a ready dispersal agent while nestled among protective spines. Dell (1965) suggests that the epifaunal habits of some small Antarctic organisms on motile hosts is related to the "lack of motile larval stages" (Dell, 1965). The lack of other hard substrata must also be a factor in deeper waters.

According to Fretter and Graham (1964), dioecious species of molluscs often have more females than males. They suggest this is related to males dying at a younger age or size. This could be the case in *Lissarca notorcadensis* but, like several other small brooding bivalves, such as *Tranzenella tantilla* (Kabat, 1985; Asson-Batres, 1988), *L. notorcadensis* could be a protandric hermaphrodite. Evidence for this includes the comparatively larger females found in the populations examined and the cross-over at around 4.0 mm height (probably more than coincidentally also the size range where we located several simultaneous hermaphrodites) from male to female dominated populations. *T. tantilla* show male dominance up to a size of 3.5 mm with females ranging from 3.5 - 7.0 mm in length (Kabat, 1985). It is possible that all specimens found on a single spine are members of the same family line. This, of course, could limit outbreeding. Protandry would, on the other hand, increase reproductive success by insuring availability of members of both sexes.

Ockelmann and Muus (1978) found that several species of the Montacutidae show reverse geotactic responses just prior to release of their larval brood. Thus, for example, *Mysella bidentata* (Montagu), a normally infaunal bivalve, becomes negatively geotactic and will emerge from the sediment and crawl upward upon available vertically oriented objects. Upon reaching the highest point available, the veliger

brood is released. Ockelmann and Muus (1978) suggest this behavior is stimulated by a "specific substance released by the young when they become ready to begin life on their own." Most specimens of *Lissarca notorcadensis*, including reproductively active adults, occur on the lower portions of the host spines. There are two likely explanations for this relationship. First, there could be an inherent or external stimulus that attracts males and females to the lower third of the spine. Extrinsically there could be a stimulus released by the surface of the urchin that attracts males and mature females or perhaps these specific bivalves are positively geotactic. On the other hand, there could be an intrinsic stimulus, such as endocrine or hormonal shifts, that are activated when either males or females reach maturity and these chemical shifts stimulate a downward migration. Similarly, one sex or stage could preferentially react to stimuli indicated above and in turn "attract" the other sex through an allelochemic interaction.

A second possible explanation is that males preferentially remain near the spine's bottom third and, being protandric hermaphrodites, a conversion of sex would result in a preferential appearance of more mature females along the spine base as well. The positioning of mature males and females along the basal third of the spine would, of course, promote successful fertilization. Brooding females are not preferentially clumped along any region of the spine. This could be indicative that brood are released along the length of the spine as a result of an upward migration of many of the brooding females after fertilization near the spine's base.

There is also an apparent seasonal influence in terms of sex dominance. As noted in the results, there is a transition of dominance from close to 60% male in May and June 1975 to about 40% in January 1975 and 45% on 12 May 1975 at a very different site. By eliminating the 12 May 1975 sample, which is situated close to the north side of South Georgia Islands (see Fig. 2) thus strongly influenced by this land mass, certain trends relegated to an austral summer and spring become clearer (Fig. 12). According to Dell (1972) the South Georgia area represents a distinct biogeographic subdivision of the Antarctic Region. This site is north of the Antarctic convergence and represents a milder climate (see Simpson, 1976). In fact, South Georgia is the northernmost distribution limit for many Antarctic species (Dell, 1965) and the northern side of the island is especially productive (DeWitt *et al.*, 1976). Thus this single site of the four sampled is placed in the South Georgian District (Dell, 1972) and results of a reproductive ecology study might be expected to differ from the other three. Another interesting difference between the two May samples is in size class distribution. The 12 May sample, again from South Georgia, contained no specimens larger than 4.5 mm in length. All other samples had larger bivalves and that from 31 May 1975, in fact, had about 20% of its population longer than 4.5 mm. It is again possible that the less stable and more seasonal South Georgia site could yield bivalves that grow slower. What is more likely is that this population is on the distributional fringe for this species and thus is subject to an earlier demise. The other three sample sites are placed in the Continental Antarctic Division

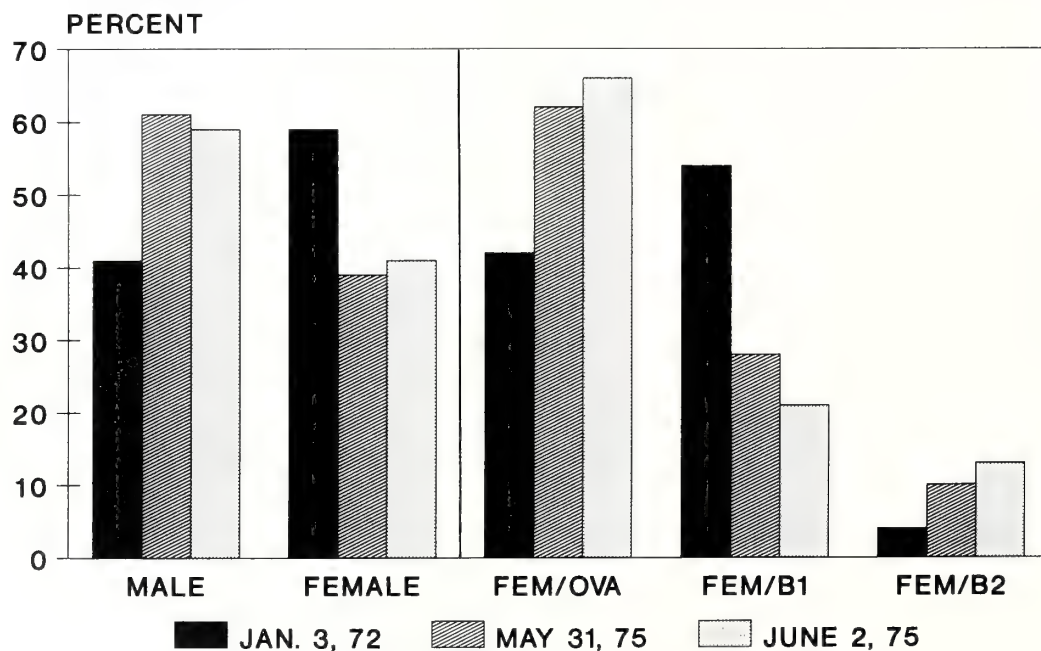


Fig. 12. Removing the 12 May 1975 (South Georgia Island) sample, more easily shows specific trends outlined in text. Again, female stages to right of vertical line represent percent of total female population only. Population numbers as per figure 4.

(Dell, 1972). The 31 May and 2 June 1975 samples are very similar to each other and clearly different from January 1972. This holds true for all stages sampled (Fig. 4).

An additional difference of some note is that of total size class distribution between 12 and 31 May 1975 and 3 January 1972. Specimens sampled on 12 May 1975 show a unimodal distribution with most specimens being between 2.6 and 4.0 mm in height. The January 1972 sample shows a similar distribution. The sample from 31 May 1975, however, has a more uniform distribution with a strong indication of bimodality (see Fig. 5). We have little information on growth rates for any philobryid. Extrapolating from commarginal lines and incremental growth measurements, Richardson (1979) suggested that *Lissarca miliaris* deposited growth rings slowly, with only one ring deposited in 3 - 15 month old clams. Indeed, Nicol (1966) suggested that, along with small size of Antarctic bivalves, these organisms are slow growing and this is indicated by numerous, narrowly spaced growth rings. Although *L. notorcadensis* is almost certainly slow growing, with growth rings already deposited in its dissoconch shell prior to release from the brood, it is difficult to extend the measurements suggested by Richardson to this philobryid. A recent review by Bourget *et al.* (1991), in fact, casts doubt on many reports that use shell growth marks to determine or substantiate bivalve age.

The differences and similarities in population size classes between *Lissarca notorcadensis* and *L. miliaris* are perhaps reflective of temporal and environmental differences as discussed elsewhere in this paper. At this point it is uncertain if the bimodal distribution represents a failed reproductive event or a natural depression in seasonal reproduction in an iteroparous bivalve reproducing more than once per year.

The philobryids are a relatively speciose and common component of the Antarctic malacofauna and yet there is very little information available on their reproductive ecology aside from Richardson's (1979). Data have been extracted from that paper for comparative purposes and are outlined on Table 2. Most importantly, any comparison drawn between these two species must be done in light of the fact that Richardson used a population of philobryids attached to a brown alga from waters less than 10 m in depth (although this species can reach depths to 121 m). Specimens of *L. notorcadensis* examined here are from waters no shallower than 110 m (this philobryid has been collected from depths as deep as 1000 m). Ice scouring is an important factor controlling shallow water fauna of Antarctica down to depths of 15 m (Moss, 1988). *L. miliaris* is restricted to protected embayments of the near sublittoral zone. Additionally, Richardson's samples of *L. miliaris* were obtained from within the bounds of the Weddell Drift, a highly productive zone of the Southern Ocean (Moss, 1988).

The two philobryids attain approximately the same maximum size although *Lissarca miliaris* reaches sexual

maturity at a slightly smaller size than *L. notorcadensis*. Additionally, *L. notorcadensis* is larger when it reaches brooding size. In the more heterogeneous shallow waters inhabited by *L. miliaris*, it might be predicted that fecundity would be higher. *L. miliaris* broods 54 to 70 juveniles, directly increasing in number with adult size. *L. notorcadensis*, on the other hand, broods a consistently smaller number regardless of adult size. Size of juveniles at release reflects the differences in brood number. Pre-release size for juveniles of *L. miliaris* is half that of *L. notorcadensis*. This of course reflects specifically the volume available for brood in the two species and the number of young able to be packed into the infrabranchial chamber.

There appears to be no size difference between males and females of *Lissarca miliaris* while this varies in *L. notorcadensis*. In the latter, females reach a larger size than males, perhaps reflecting protandry as discussed above. It is clear that brooding females in *L. notorcadensis* are larger than males as well as sexually mature but nonbrooding females.

The general life cycles of the two species have some basic similarities in terms of development but there are important temporal differences noted in the populations examined. Preshelled young are evident from February to July in *L. miliaris* but likely dominate in the austral summer for *L. notorcadensis*. In the latter species, however, some preshelled brood are evident in all samples examined. Fully shelled juveniles dominate from November to December in *L. miliaris* and can be found at some level in all our samples of *L. notorcadensis* but are presumed dominant in May and almost absent in January. Recruitment for *L. miliaris* and *L. notorcadensis* is from December to February and likely June through December respectively. The latter significant time shift again seems to reflect habitat differences in populations studied. Pearse *et al.* (1991) review the contrast in the "physically unstable shallow benthos" and the "physically stable deeper benthos". In oligotrophic waters deeper than 20 - 30 m many benthic invertebrates produce pelagic lecithotrophic larvae that yield larger juveniles more resistant to predation pressures. *L. notorcadensis* extends this to release of brooded juveniles that are not only large but protected by the host urchin. As *L. notorcadensis* is one of the most common molluscs of the antarctic region, the success of this mode of development seems apparent. Grahame and Branch (1985) review different modes of reproduction in marine invertebrates and recognize that Antarctica represents an intriguing case where primary production and potential paucity of food could regulate reproductive strategies. They suggest that more information on these south polar organisms is needed to fully appreciate their modes of reproduction. While we are able to discern much information through the use of museum material, to fully appreciate the unique evolutionary game plans developed by these small brooding molluscs, we must promote longer term studies of the living organisms.

ACKNOWLEDGMENTS

Specimens for this research were kindly loaned by the Smithsonian Oceanographic Sorting Center. The assistance of G. Hendler, J. Norenberg, M. Fissler and J. Houbick are particularly noted in this loan. Additional assistance in the quantitative aspects of this research were supplied by K. Snyder, S. Magaro and S. Logan, and we thank them for their efforts. Many thanks to J. H. Dearborn for helpful information on Antarctic cruises and echinoderms. Thanks also to Mr. Doug Shumar for drafting figures 2, 7 and 8. We appreciate the efforts of two anonymous reviewers who helped to clarify the text and refine some of our ideas. This research was funded by a Faculty Research Award to RSP of Indiana University of Pennsylvania.

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Date of manuscript acceptance: 28 February 1992

The evolution of the hindgut of the deep-sea protobranch bivalves

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Abstract. The evolution of the hindgut of the deep-sea Protobranchia is considered in relation to their feeding strategy. One of the cornerstones of their success in colonizing soft abyssal sediments lies in their ability to digest organic materials extracellularly in relatively low concentration. The time needed to digest such materials contained in a continuous column of sediment in the hindgut is maximized by a much elongated gut. Elongation has been accompanied by a variety of configurations in its course, these maximize the length of the tube that can be housed within the space of the visceral mass. It would appear that this has been a major evolutionary concern, if not the major evolutionary concern, of the deep-sea protobranchs and has proved to be of considerable taxonomic importance.

The Palaeotaxodonta, which comprise the vast majority of the deep-sea (>500m) protobranchs, are all deposit feeders and commonly as many as fourteen species can be collected at a single station both on the lower slope and the abyssal plain (unpub. data). Analysis of the gut contents of specimens representing various species from a single station indicates that all are consuming a similar fraction of the soft sediments. Most, if not all, feed via their palp proboscides in the surface layers of the abyssal sediments and, probably for the most part, on the surface layer itself. In such a situation it is natural to speculate what, if anything, separates the ecological niches of the cohabiting species. In terms of Hutchinsonian Ecology, Allen (1985) has suggested that habit and space could be the separating factors. A more extreme view would be that there is no niche separation, that competition is low and that speciation is neither driven nor restricted by stress. For instance, high pressure could be considered stressful, however having become adapted, in reality all physical factors of deep-sea basins are stable and predictable over geologic time and despite being extreme are unlikely to be stressful. Animal density is low and there is circumstantial evidence that predation is remarkably low (Oliver and Allen, 1980; Turekian *et al.*, 1975), but this may or may not be an indication of the level of competition. Diversity is high however measured (Sanders and Hessler, 1969) with a range of frequency of occurrence of species from common to rare. Community structure in the deep sea is not atypical compared with other marine communities. Apart from indications from morphology of small variations in habit and function (i.e. some species may be less mobile and deeper burrowing than others) it must be concluded that we are as yet largely blind to the delineation of niche for the infaunal protobranchs of the deep sea.

THE PROTOBRANCH GUT

There is one morphological variation that is particularly obvious in the Palaeotaxodonta, namely the configuration of the hindgut. In naive terms it could be concluded that in their evolution the main thrust has been how best to accommodate an elongate hindgut within a small body space rather than changes in external shell morphology to gain ecological advantage in a monotonous environment. External morphological variations are subtle, often to the extent that one taxon can be barely recognized from another (Allen and Hannah, 1986).

Numerically the protobranchs dominate the particulate feeding abyssal bivalve fauna (Clarke, 1962; Sanders *et al.*, 1965). (Note: Carnivorous septibranchs are well represented in the deep sea.) One thing, possibly the only one, that explains this dominance is a difference in the digestive physiology of the protobranchs as compared with the lamellibranchs. Little is known of the digestive processes in protobranchs but what would indicate a major difference in the function of the digestive diverticula (Owen, 1956, 1980; pers. obs.) as compared with that of lamellibranchs. In the organically rich sediments and water column of shelf seas this difference could be of little significance, but in the relatively impoverished sediments and water column of the abyss (Gage and Taylor, 1991), digestive efficiency is vitally important. The efficiency of the selective process to extract scarce organics within a slurry of fine sediment, together with the time taken to digest skeletal proteins also becomes important.

To maximize extracellular digestion of refractive food material in relatively low concentrations in fine sediments, and the material attached to silt particles, requires an

increased residence time within the gut. An elongated hindgut allows for greater passage time of sediment and a larger surface of contact between digestive epithelium and gut content, allowing for a more complete digestive and adsorptive process. The elongate gut has to accommodate in a small and confined space and this has been accomplished in a variety of ways. Also, an efficient method of transporting a very long column of sediment through a narrow pipe had to be achieved.

To address the last point first, movement through the gut of protobranchs could be dependent, at least in part, on the use of the stomach and the style sac as a squeezable bulb, the pressure so created driving the fluidized gut contents. This is in contrast to the lamellibranchs in which ciliary transport appears to be the driving force. Anatomical observations indicate that pressure can be exerted in two ways. One is via the surrounding musculature basal to the stomach epithelium and the second is via modified anterior pedal retractors, which cradle the ventral side of the stomach (unpub. data). When contracted the latter squeeze the dorsal side of the stomach against the inner shell below the umbo. In the protobranchs the dorsal surface of the stomach lies immediately adjacent to the epithelium of the body wall that lines the shell.

THE HINDGUT OF THE NUCULANOIDEA

The basic design of the hindgut of the nukulanoideans, the most common of the deep-sea protobranchs, is a loop on the right side of the visceral mass (Fig. 1a). The loop extends from the ventral limit of the style sac dorsally posterior to the style sac and stomach, and thence on the right side in a sweeping antero-ventral curve to the mouth and anterior adductor muscle and from there dorsally and posteriorly along the dorsal side of the viscera, passing through the heart and dorsal to the kidney and posterior adductor muscle to the anus. In most species a single typhlosole is present along the whole length of the hindgut.

From this simple configuration a limited number of different types of more complex configurations can be derived. The range of types of configuration is present in a number of families or subfamilies and these types could have evolved more than once in the history of the group.

Although there are exceptions, in general protobranch species with more elongate hindguts are found deeper in the ocean than those with short hindguts. For example, *Tindaria callistiformis* Verrill and Bush, 1897, from depths between 3305 and 5042m, has a hindgut volume per unit animal that is half as large again as *T. hessleri* Sanders and Allen, 1977, from depths between 1739 and 2339m (Sanders and Allen, 1977). More recent measurements of length show that this applies to other protobranch genera, thus *Yoldiella subcircularis* Odhner, 1960, from 3250-5987m, has a hindgut that is more than four times the length of *Y. inconspicua* Verrill and Bush, 1898, from 1102-4829m, in animals of the same size

(pers. obs.). The protobranchs are not alone in showing this phenomenon, thus similar increases in gut length with depth were shown in the case of a series of species of the tellinoidean genus *Abra* living at increasing depths (Allen and Sanders, 1966). In many species it would appear that the extended single loop simply takes a wider course around the right side of the body. The diameter of the lumen of the hindgut may also be increased. As seen in the tindariids (Sanders and Allen, 1977) the lengthening of the single loop at its most extreme involves the penetration of the visceral tissue containing the ventral portion of the loop into the mantle of the right side to lie close to the inner muscular fold of the mantle edge. (Fig. 1B). In less extreme cases the ventral limit of the loop lies at the margin of a visceral fold that in large part overlies the right palp. In most cases the lengthened hindgut also penetrates deep into the foot ventral to, and in some cases, anterior to the pedal ganglia. The configuration of the path taken and the diameter of the gut is quite specific and in very many cases the identification of the species can be accurately based on this one feature.

In the progression from a single loop, one sequence involves the coiling of the loop on the right side. This process can be easily illustrated by manipulating a length of rope and taking hold of the anterior limit of a loop and coiling once, twice and eventually more times in a clockwise direction. Individual species have their own characteristic form and number of coils (Figs. 2A-F). Multiple coils have sufficient volume to cause displacement of the stomach to the left, and to limit the digestive diverticulum of the right side to the centre of the coil (Allen and Hannah, 1989).

The second sequence from a single loop is but a variation of that described above. Here the anterior section of the loop extends to the left side of the body passing between the oesophagus and the anterior adductor muscle. On the left side of the body a loop is formed and this, depending on the species, may or may not be coiled in the manner described above (Figs. 2G, H). The morphological consequence of this penetration to the left side of the viscera is to displace the mouth posteriorly. This latter form of hindgut configuration is confined to relatively few species.

Much more common is a third sequence in which the hindgut on reaching the dorsal margin of the visceral mass to the stomach passes first to the left side of the body, makes a loop around the periphery of the visceral mass, then passes to the right side of the body and describes a single loop of similar extent to that on the left (Fig. 3A). Various extensions and modifications to this left and right looped configuration occur in different species. The most common is where right and left loops are doubled on each side. In rarer cases additional loops can be formed on either side. In species with a vertically elongate foot, lengths of gut can be doubled or quadrupled parallel to the style sac and reach far ventral within the foot (Figs. 3B, C).

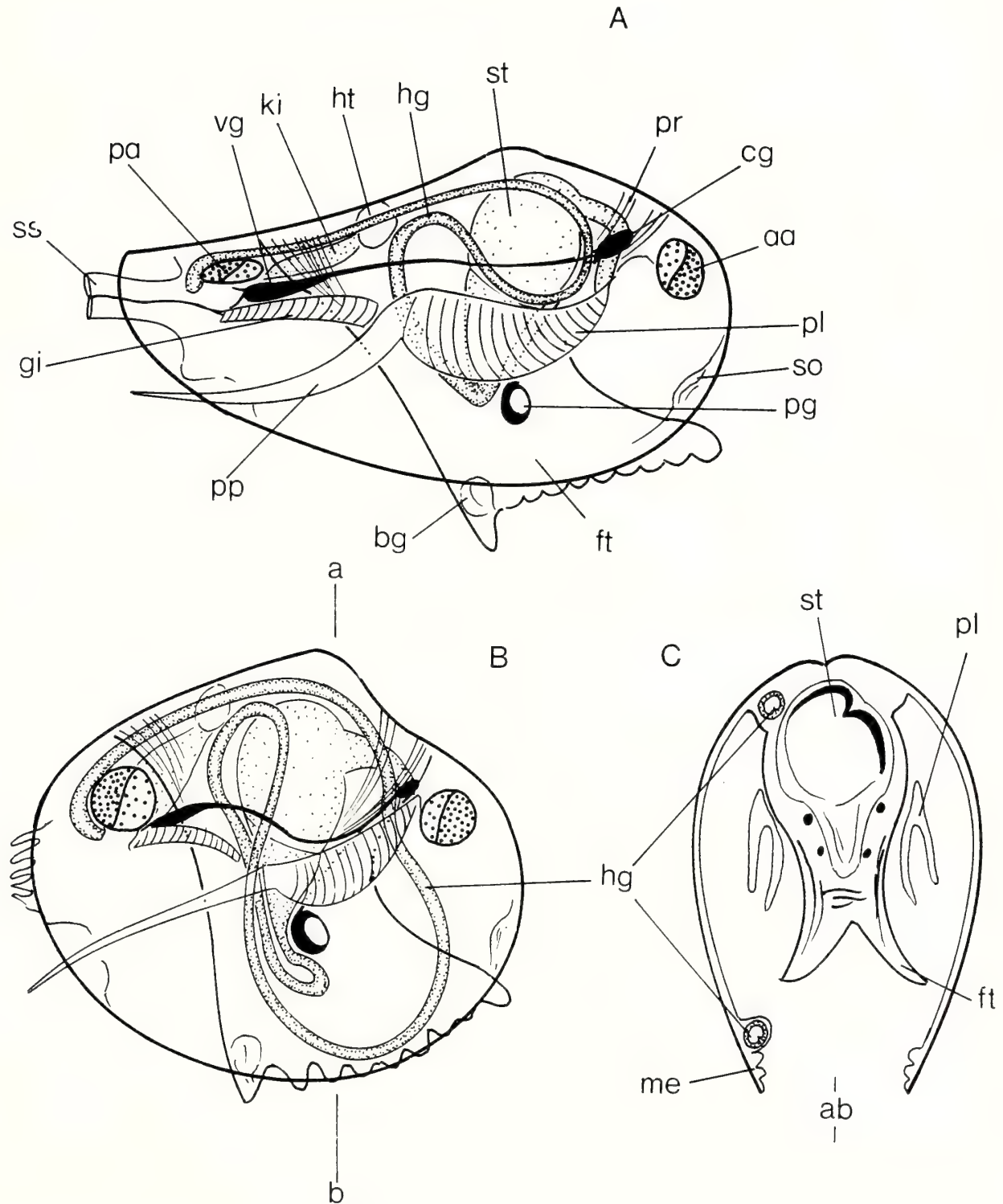


Fig. 1. A, A diagrammatic representation of a generalized nudibranch from the right side to show the major morphology of the body organs; B, a similar diagrammatic representation of a tindariid nudibranch as seen from the right side (for identification of the organs see (A)); C, a diagrammatic representation of a vertical cross-section of a tindariid nudibranch through the lines a-b in (B) to show the position of the hindgut. aa, anterior adductor muscle; bg, byssal gland; cg, cerebral ganglion; ft, foot; gi, gill; hg, hindgut; ht, heart; ki, kidney; me, mantle edge; pa, posterior adductor muscle; pg, pedal ganglion; pl, palp; pp, palp proboscides; pr, pedal retractor muscle; so, anterior sense organ; ss, siphons; st, stomach; vg, visceral ganglion.

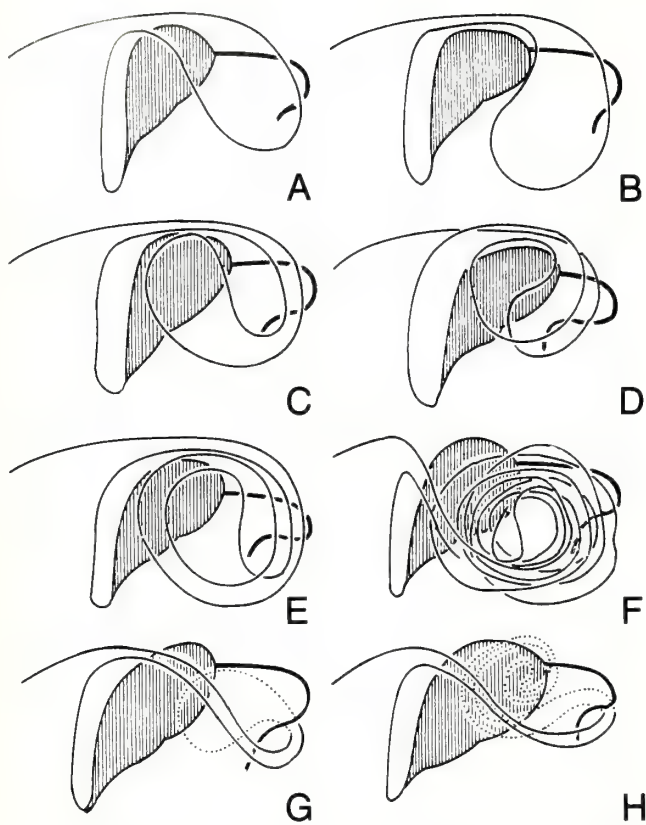


Fig. 2. Line drawings of the course of the gut in eight species of *Yoldiella* to illustrate the different courses taken by the hindgut with increasing length of the hindgut. The drawings are from the right side, the end of the line to the left represents the anus; the end of the curved line to the right, the mouth; the stomach is shown as a 'pear'-shaped outline. The course of the hindgut is shown as a solid line on the right side of the body and as a dotted line on the left.

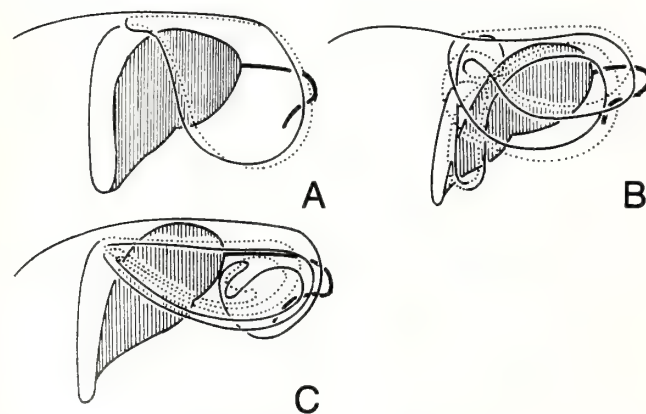


Fig. 3. Line drawings of the different courses taken by the hindgut of three species of *Yoldiella* in which the hindgut loops to both the left and right of the body. The course on the left side is differentiated by a dotted line. Note in all these species the point of passing from left to right and from right to left is posterior to the postero-dorsal limit of the stomach.

In all of the configurations of the second and third sequences described above, the passage of the hindgut from one side of the body to the other occurs either anteriorly between oesophagus and anterior adductor muscle or mid-dorsally immediately posterior to the stomach. It can be pictured that, as the hindgut develops, it is these two points that provide the gateway to the extension to the left side of the body. It is of note that in no species as yet seen does penetration to the left occur at *both* points in an individual species.

In relatively few species penetration of the hindgut to the left side of the body occurs both postero-dorsal to the stomach and *anterior* to the stomach (but posterior to the oesophagus) within the mass of the viscera. As in the second and third sequences this has occurred in well-separated taxonomic groups (Lametilidae and Yoldiellinae) (Allen and Sanders, 1973, unpub. data).

THE HINDGUT OF THE NUCULOIDEA

In the case of the Nuculoideans, no species has yet been described in which there is a hindgut that describes a single loop to the right. Nevertheless, there is much conformity within the Nuculoidean taxa. Thus, the Nuculidae all have a coiled hindgut predominantly dorsal and inclined to the right of the stomach, while the Pristiglomidae have multiple loops to the right and the left passing from side to side postero-dorsal to the stomach but with loops carried dorsal to the stomach and viscera (Sanders and Allen, 1978; Rhind and Allen, 1992).

DISCUSSION

The disposition of the hindgut provides additional clear evidence of the differentiation of the Nuculoidea into the Nuculoidea and Nuculanoidea - already well-established on other morphological and functional grounds (e.g. the presence or absence of an anterior inhalent current, anterior mantle sense organ and posterior inhalent and feeding apertures) (Fig. 4). Hindgut differences clearly differentiate the two nuculoidean families, the Nuculidae and the Pristiglomidae.

Similar universal differences cannot be seen in the case of the nuculanoideans. In a few families there is a predominant hindgut configuration (Fig. 4). These more clear-cut examples include the Nuculaninae, which possess a single loop to the right that is not particularly lengthened, and the Malletiidae and the Tindariidae in which the single loop is extended (Sanders and Allen, 1977, 1985). In the latter family the hindgut is usually carried into the right mantle lobe. In the Spinulinae, multiple coils are present on the right side of the body in most species (Allen and Sanders, 1982). In contrast, species of the Ledellinae (Allen and Hannah, 1989), the Yoldiellinae and the Neilonellidae (unpub. data) exhibit a wide range of configurations. Whether or not these groups

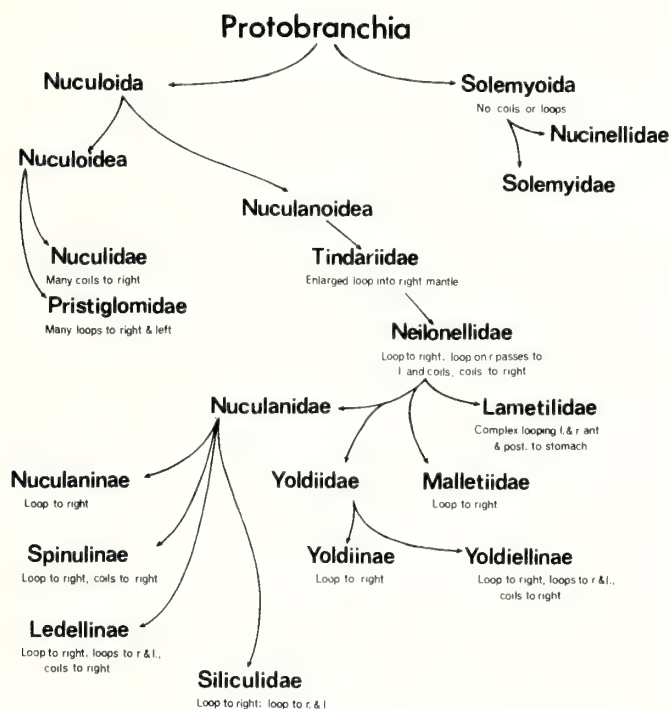


Fig. 4. Possible evolutionary relationships within the Protobranchia.

are discrete entities or whether they are polyphyletic is not yet absolutely certain. In the case of the Ledellinae, the subfamily comprises a series of closely related species in which other characters, particularly those of the shell, show not only close relationship but are sufficiently distinct as to delineate a suprageneric taxon. This is less clear in the Yoldiellinae (unpub. data), which comprises a very large number of species, but without the strongly marked shell characters displayed by the Spinulinae and Ledellinae. Overall the differences in shell characters are so slight as to be impossible to arrange the species into distinct units. The Neilonellidae are presently under review, but the indications are that this family exhibiting a variety of gut configurations is monophyletic (pers. obs.).

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Date of manuscript acceptance: 20 April 1992

Prismatic shell formation in continuously isolated (*Mytilus edulis*) and periodically exposed (*Crassostrea virginica*) extrapallial spaces: explicable by the same concept?

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Abstract. Many aspects of molluscan biomineralization and shell formation yet remain unexplained. A comparison of formation of prismatic shell in two species of bivalves is presented that suggests, as indicated by a current hypothesis, that soluble organic matrix precedes and regulates the type and form of biocrystals. In *Mytilus edulis* Linné prisms develop at the shell margin in an extrapallial space continuously closed to seawater by the emerging periostracal sheet, the mantle margin remaining in place. In *Crassostrea virginica* (Gmelin), however, margins of mantle lobes are frequently withdrawn into the mantle cavity, exposing prismatic shell surfaces to seawater. Prism-secreting mantle cells, even though they could be precisely repositioned over growing prisms, probably do not control the growth of these prisms; rather, once nucleated in the soluble matrix, each biocrystal with its accompanying matrix probably mediates mineralization and shape and size of that prism and subsequent prisms in the prismatic column. Thus, after the secreting epithelium is extended to the valve edge, bathing growing prisms in extrapallial fluid, development is resumed without deformation.

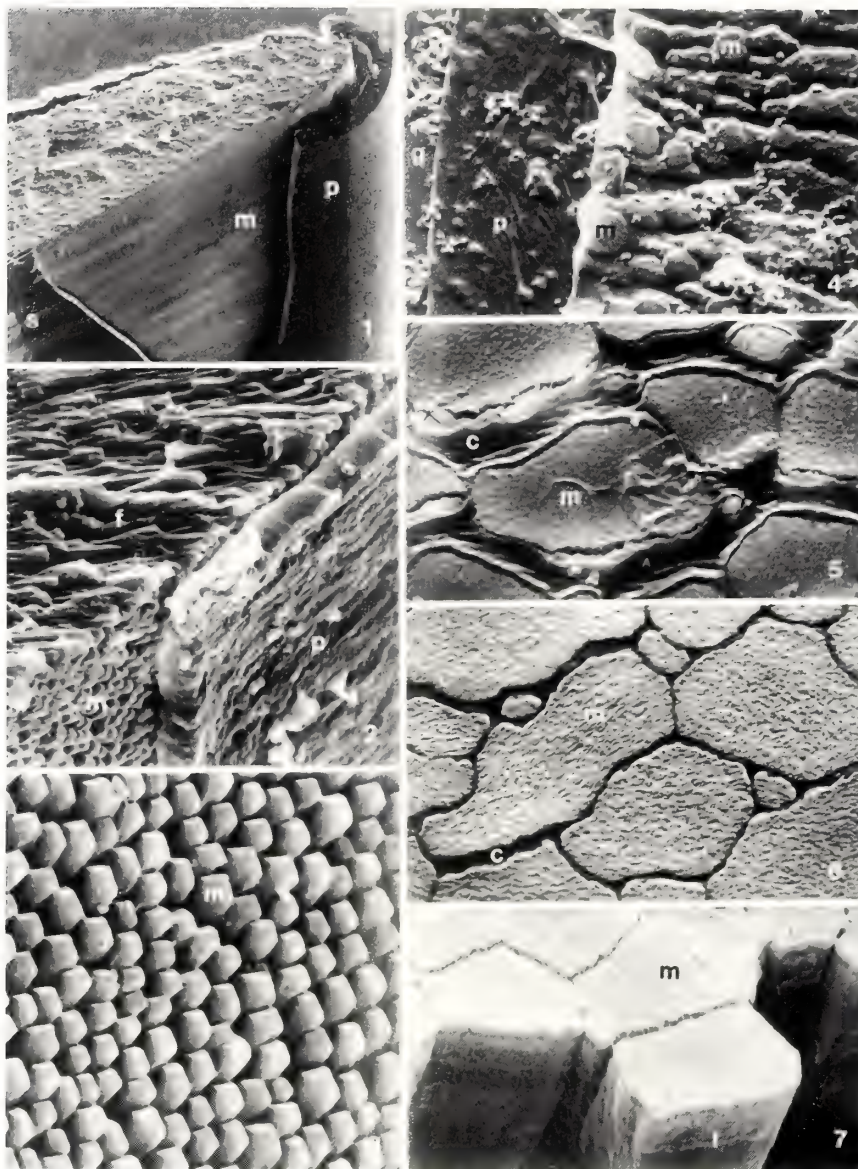
Studies of the morphology and development of molluscan shell have proliferated during the past thirty years. Yet many aspects of shell formation, such as biomineralization, for example, remain imperfectly understood. Of primary interest are the cellular physicochemical mechanisms that bring about nucleation, orientation, size, micromorphology, and polymorphic type of the biocrystals, the basic microstructural units of shell (Simkiss and Wilbur, 1989; Carriker, 1991; Rosenberg and Hughes, 1991). The present overview explores principally the micromorphological interplay at bivalve shell margins of a) the shell-secreting epithelium, b) developing prismatic microstructures, and c) freshly secreted periostracum in a) an extrapallial space continuously closed to seawater, and b) one periodically exposed to flooding by seawater.

Is it conceivable that prismatic microstructures, formed in two such apparently disparate anatomical microenvironments, are the products of the same biophysicochemical mechanism? It would appear so. The existence of such a similar mechanism is indicated by the present comparison of formation of prismatic shell in two species of molluscan bivalves, both in the Subclass Pteriomorpha: *Mytilus edulis* Linné (Family Mytilidae, Order Mytiloida) and *Crassostrea virginica* (Gmelin) (Family Ostreidae, Order Pterioidea). The mechanism is interpretable in terms of the current hypothesis that organic matrix of shell is an organized medium that serves as a mediator of biomineralization (Simkiss and Wilbur, 1989) in the extrapallial space.

EXTRAPALLIAL SPACE

Among bivalve molluscs, extracellular shell formation generally occurs between the mantle shell-secreting epithelium and the inner surface of each of the two shell valves in the very thin extrapallial space. Within this space, the solubility product of the minerals being incorporated in shell microstructures is probably surpassed by a change in the concentration of precursor ions. The distance between epithelial and shell surfaces is so small that transfer of mineral ions and organic molecules from the secretory epithelium to the valve surface could occur virtually by direct contact (Simkiss and Wilbur, 1989).

In many bivalves the extrapallial space is closely sealed from seawater. This closure is effected by the newly emerging periostracal sheet, which maintains contact between the outer mantle margin and the free edge of the valve (Saleuddin and Petit, 1983). As the periostracal sheet is pressed from the periostracal groove, it becomes temporarily attached to the outer surface of the middle mantle fold by adhesive epithelia. Blood pressure in the hemocoel of the outer mantle fold holds the secreting epithelium tightly against the periostracum (Dunachie, 1962-63). In this way, the extrapallial space is completely enclosed, and shell growth at the valve margin takes place without dilution by environmental seawater. Species with continuously isolated extrapallial spaces include the marine bivalve *Mytilus edulis* (Figs. 1-3) (Dunachie, 1962-63; Mutvei, 1972; Bayne, 1976; Rosenberg



Figs. 1-3. Scanning electron micrographs (SEM) of valves (6 cm long) of the shell of *Mytilus edulis*, washed, air dried, fractured with a hammer, pieces mounted and coated with gold. **Fig. 1.** A fractured surface (f) perpendicular to prismatic shell margin (e) in mid-ventral region of the valve, showing the edge enveloped by periostracal sheet (p). Inner edge of sheet (in life arising in the periostracal groove of the mantle edge) now stuck to inner surface of the valve(s). Growing prisms (m) lie inside periostracal sheet (p) (horizontal field width = 0.9 μ m). **Fig. 2.** Enlargement of figure 1 at outer edge (right) of the V-shaped break in periostracal sheet (p), prism tips (m) to the left beneath it. Fracture surface (f) [horizontal field width (hfw) = 45 μ m]. **Fig. 3.** Enlargement of prism ends (m) in figure 1 (hfw = 18 μ m). **Fig. 4.** SEM of clear, new, single, dried, periostracal sheet (p) slightly folded under (to the left), older part of the sheet (to the right) containing a small developing prism (m), at the mid ventral edge of the left valve of a spat of *Crassostrea virginica* (1.2 cm high) that had set on a glass surface (g); oyster flesh was pulled away leaving periostracal sheet in place against the glass surface. Specimen was dehydrated in alcohol, mounted, oven dried, and coated with carbon and gold (hfw = 20 μ m). **Figs. 5-6.** SEMs of inner surface of prismatic shell of *C. virginica* raised in a hatchery until 1 cm high, then grown in local estuary for 2 months, and finally laboratory cultured where food was added and seawater was changed daily for 10 days to allow oysters to deposit shell in undisturbed conditions. Valve surfaces were cleaned with a soft brush under tap water, pieces were sawed out under running tap water to avoid contamination with shell dust, dehydrated in alcohol, mounted, and coated with carbon and gold. **Fig. 5.** Interior surface of the mid-ventral prismatic margin of right valve, a short distance inward from valve edge. Prisms (m) are considerably larger than those in figure 4, and there is still substantial organic matrix (c) (concentrated) between prisms (hfw = 40 μ m). **Fig. 6.** The same general region of interior surface of prismatic margin as in figure 5 but farther inside the shell edge. Prisms are larger, and have "crowded out" more of the organic matrix (c) (hfw = 40 μ m). **Fig. 7.** SEM of three dimensional view of young full formed prisms (m) in a section of right valve of *Crassostrea virginica* (same lot as in figures 5-6) fractured by breaking and showing growth layers (l). The preparation was treated with full strength bleach for 1 min before dehydration to dissolve superficial organic matrix (hfw = 40 μ m).

and Hughes (1991), the freshwater clam *Amblema plicata perplicata* (Conrad) (Saleuddin and Petit, 1983), and the freshwater and estuarine invader *Dreissena polymorpha* (Pallas) (Morton, 1960).

In a second major group of bivalve molluscs, pallial attachment to the interior surface of the valves occurs well inside valve margins, allowing deep retraction of the ventral half of the mantle lobes and flooding of extrapallial spaces with seawater. Mantle withdrawal is characteristic, for example, of species of the Anomiodea, Limoidea, Ostreodea, Pectinoidea, and Pinnacoidea. In these taxa, structural changes associated with monomyarianism (Yonge, 1953) have resulted in secondary pallial attachment, tenuous mantle-periostracal contact, and a thin, inconspicuous external shell periostracum (Taylor *et al.*, 1969). A notable example of a species that periodically exposes its extrapallial spaces is the Eastern Oyster *Crassostrea virginica* (Galtsoff, 1964; Carriker *et al.*, 1980; Carriker, 1991). Another is the San Diego Scallop *Pecten diegensis* Dall (Clark, 1974).

In species with periodically exposed extrapallial spaces, the margin of the valves tapers to a thin edge, and the forming periostracum arising in the periostracal groove is extremely thin and appears to possess little stability. The region of the mantle lobes between the single adductor muscle and ventral margins can be physically highly active. In *Crassostrea virginica*, for example, in which the only attachment of the mantle lobes to the valves is at the circumference of the adductor muscle, the lobes can extend some distance beyond the edge of the valves, withdraw deeply inside the shell, form ridges, and roll into a temporary channel to facilitate rejection of undigestible particles in mucus. These movements can involve a small part of the ventral half of the lobes, or all of it, depending on the intensity of stimulation received by tentacles of the middle and inner mantle folds. In an oyster whose valves are closed, mantle margins are normally retracted to midway between the margin of the gills and edges of the valves (Galtsoff, 1964; Carriker, 1991). Mantle contraction exposes newly forming surfaces of shell to a wash with seawater, and breaks any connection that might have existed between periostracal sheets and valve edges.

ORGANIC SHELL MATRIX

Organic matrix of molluscan shell is considered an organized, genetically programmed medium that in some way functions to nucleate minerals, determine the mineral phase (polymorph), and regulate crystallographic orientation, microarchitecture, growth, and size of mineralized shell microstructures (Simkiss and Wilbur, 1989; Crenshaw, 1990). A current view is that insoluble matrix forms a structural framework, and that soluble matrix present in and around it in the extrapallial fluid serves as a nucleating surface; in solution, soluble matrix possibly can act as an inhibitor, con-

trolling the thickness of layers (lamina) of microstructures (Simkiss and Wilbur, 1989; Crenshaw, 1990).

Structurally speaking, each biocrystal originates as an ion cluster that grows into a critical nucleus attracted to, or formed at, the charged surface of the insoluble matrix. Each nucleus, upon addition of ions from the extrapallial fluid, develops into a small crystal, and this grows into a definitive biocrystal within the insoluble matrix. The biocrystal and accompanying internal and external soluble and insoluble matrix constitute the microstructural unit. It is possible there is feedback from the growing biocrystal surface to secretory mantle cells over the biocrystal that could facilitate coordination of microstructural formation. Shell microstructures ultimately reach a general size and shape consistent with the morphological-mineralogical type characteristic of its shell region (Wilbur, 1974; Carriker *et al.*, 1980; Simkiss and Wilbur, 1989; Crenshaw, 1990; Carriker, 1991).

Composition of the insoluble organic matrix varies in different microstructural regions of a single valve (Simkiss and Wilbur, 1989; Crenshaw, 1990), probably accounting, in part, for such dissimilar microstructures as prismatic, foliated, chalky, and myostracal in, for example, *Crassostrea virginica*.

PRISM FORMATION IN AN OPEN EXTRAPALLIAL SPACE

A closer examination of how prisms appear to form at the growing margin of the valves of *Crassostrea virginica* explains how the developing concepts of microstructural formation reviewed in the previous paragraphs could apply, not only in isolated, but also in periodically exposed extrapallial spaces.

Mantle-edge activity and formation of shell prisms at the margin of valves have been studied a) in young live oysters in seawater under a binocular microscope (Tomaszewski, 1982), b) by inserting pieces of thin glass between the edge of the mantle and the valve of live oysters, and removing the glass at regular intervals for analysis under the light microscope (Galtsoff, 1964), and c) viewing with a scanning electron microscope the newly deposited periostracum and developing biocrystals at the edge of the left valve of young oysters that had set on small pieces of thin glass (Carriker *et al.*, 1980; Carriker, 1991). Clark (1974) investigated the growing margin of *Pecten diegensis* with time lapse photography and scanning electron microscopy.

During prism formation, the oyster, valves gaping slightly and pumping seawater, extends both left and right mantle-lobe margins a short distance beyond their respective, thin, mineralized, valve edges. In the temporarily enclosed extrapallial space of each valve, the oyster releases onto and beyond the mineralized valve margins from the periostracal grooves, a thin, clear, viscous, sometimes stringy, sheet of

periostracum. When the margin of the left valve is affixed to a hard substratum (like shell or glass), the new periostracal sheet is laid over this (Fig. 4); the sheet on the right valve if off of the substratum and that on the left valve, if the valve is no longer attached to the substratum, are extended into and remain suspended in seawater. Contact with seawater probably firms the previously liquid periostracal sheets enough that they maintain their form and position even though suspended in seawater. During release of the fluid periostracum, the mantle expands and retracts actively, spreading the secretion over previously deposited periostracal layers and beyond these to form new ones. New left and right marginal periostracal sheets probably never adhere to each other during shell formation because oysters, with valves slightly apart, continue pumping seawater during shell deposition. It is likely periostracum soon loses its surface adhesiveness when exposed to seawater.

The first crystallites, becoming visible under the magnification of the scanning electron microscope as very small, roughly rounded, randomly distributed bodies (0.01 μm or less), and presumably surrounded by extrapallial fluid supersaturated with respect to the minerals being deposited, appear embedded within the new periostracal matrix, over and beyond the previously mineralized shell edge (Fig. 4). As the thin, wafer-like biocrystals grow in diameter and thickness, their margins approach each other, apparently "squeezing" organic matrix between them (Figs. 5, 6). Once lateral boundaries of prisms come close, prism growth becomes primarily lengthwise, resulting at maturity in long slender needle-like structures generally polygonal in cross section (Fig. 7). In micrographs, biocrystals are shown as growing embedded within the sheets of organic matrix (Fig. 4) rather than on the surface (Carriker *et al.*, 1980; Wilbur and Saleuddin, 1983; Simkiss and Wilbur, 1989).

It would seem that in the oyster initial biocrystal formation can occur virtually independent of a stable shell margin substratum, at least in those parts of the freshly formed periostracal sheet suspended in seawater beyond the edge of the mineralized shell margin. Whether, as Clark (1974) suggested for the scallop, stability on a firm foundation is a requirement for orderly shell growth in the oyster, is unclear. This seems unlikely in view of the sequence of prism development represented in the micrographs (Figs. 4-6).

Being exposed to environmental seawater during periods of withdrawal of the mantle, the freshly growing, apparently slightly viscid marginal valve surface could be vulnerable to contamination by clays, silts, and other suspended particles. This could explain the presence of foreign particle and chemical contaminants in shell (Simkiss, 1965; Carriker *et al.*, 1982, 1991). The degree of "stickiness", if any, of the hardening periostracum has not been determined. Adventitious impurities are probably characteristically present in all molluscan shells that possess transiently open ex-

trapallial spaces, and especially of those species that inhabit coastal and estuarine waters that tend to be highly turbid (Carriker, 1967; Carriker *et al.*, 1980; Carriker, 1986).

COMMON MECHANISMS AND FURTHER INQUIRIES

Enclosed, continuously isolated extrapallial spaces, like those of *Mytilus edulis*, would seem to offer an ideal microenvironment for the growth of prismatic biocrystals (Figs. 1-3). By contrast, periodic withdrawal of mantle lobes and the ensuing flooding of extrapallial spaces with seawater, as in such species as *Crassostrea virginica* (Figs. 4-6), would appear to pose a disadvantage in the process of marginal prism formation.

Yet, this strikes me as not being the case, because bivalves with open biomineralization systems form shell apparently as successfully as those with closed systems; both groups contain species that are probably equally successful biologically. One can conjecture that in the Bivalvia, biomineralization evolved first as an extracellular process on open mantle edges (Stasek, 1972). Hence evolution of the genetically programmed organic matrix of shell appeared first in open systems, and was retained as a basic component of the biochemical mechanism of biomineralization as closed systems evolved in other species. To what extent intermediate anatomical configurations between open and closed systems, if they exist, might shed information on the evolution of closed systems, would be worth investigating (Simkiss, 1974).

What utility, if any, the closed system could have over an open one relative to the mechanism of shell formation and survival of the bivalve, is not clear. There is the obvious possibility that shell produced in isolated margins is freer of adventitious particles and chemicals than that in open margins; whether this is, or is not, an advantage to a bivalve is still a question, and a point worth pursuing in view of the increasingly contaminated waters of coastal regions of the world (Simkiss, 1965; Carriker, 1976).

Because of the hypothesized importance of the genetically programmed organic matrix, whether in open or closed mantle margins, there is no longer any need to presume, as one of the possibilities given by Carriker *et al.* (1980), that a precise spatial microassociation could exist between mantle epithelial shell-secreting cells and developing microstructural units growing apposed to them, nor that specific clusters of secretory cells must be repositioned over the ends of biocrystals first formed by them before each withdrawal of mantle lobes.

Although prismatic shell formation, or any shell growth for that matter, is understood imperfectly (Simkiss and Wilbur, 1989), even scantier knowledge is available on the control of the transition in microstructure and in mineralogy in the same developing shell valve. There are such

common changes, for example, as marginal prismatic shell to foliated (in oysters) or nacreous (in mussels) inside the valves as the shell increases in size, or the introduction of aragonitic myostracum over calcitic folia (in oysters) as the adductor muscle scar migrates marginward during shell enlargement (Wilbur and Saleuddin, 1983).

Although the basic mechanisms of biocrystal formation could be common to all biological systems (Crenshaw, 1990), as shown in *Mytilus edulis* and *Crassostrea virginica* the physicochemical microenvironment in which biomineralization occurs can vary. Comparative studies of the similarities and differences in the anatomical structures and processes of shell formation in different taxa could provide a greatly needed perspective (Wilbur, 1974). And because biomineralization is largely about ions and structures, initially at molecular and cellular levels, and subsequently at the organismal level (Simkiss and Wilbur, 1989), comparative investigations could be exceedingly fruitful when approached multidisciplinarily.

Crenshaw (1990) noted that most of the progress toward an understanding of biomineralization at all phylogenetic levels has been made as a result of research on invertebrate animal systems, especially those of molluscs. The present overview would suggest that *Mytilus edulis* and *Crassostrea virginica*, because of the strikingly different arrangement of the microspaces in which shell formation takes place in these species, would serve as excellent models in the further unravelling of the fascinating complexities of biomineralization and mineralized skeletal formation in both plants and animals.

ACKNOWLEDGMENTS

Special thanks go to Dirk Van Zant, Virginia Peters, John Ewart, Robert Palmer and Robert Prezant for collaboration in the original preparation of specimens and scanning electron microscopy. Robert Bowden II prepared prints of the micrographs. I am also grateful to Miles Crenshaw, Karolyn Mueller Hansen, Robert Prezant, Kenneth Simkiss, and Herbert Waite for valuable comments on the manuscript.

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A new approach to the study of bivalve evolution

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Abstract. Systematic and evolutionary studies of the Bivalvia have been based mostly on obvious conchological characters, but such characters could often reflect parallel adaptations and not phylogenetic relationships. Of the various biomolecular techniques capable of measuring genetic relationships among taxa, radio-immuno-assay (RIA) is particularly suited for bivalve studies. RIA measures genetic distance between taxa by measuring how much antibody made against proteins of one species binds with proteins from another. The results correlate highly with DNA hybridization, DNA sequencing and microcomplement results. RIA is unique in that it can extract information from proteins preserved in fossil and recent calcareous matrices. Because most molluscan collections consist predominantly of dry shells, RIA could prove to be very important for their evolutionary studies. I am using RIA to study parallel evolution in Veneridae and to develop a phylogenetic outline of the family. Venerid taxonomy is based currently on obvious conchological similarities. Preliminary results indicate that: 1) obvious conchological similarities can be parallel adaptations; 2) closely related species could exhibit wide conchological divergences in response to different life strategies; 3) Veneridae is deeply divided, but its origin is monophyletic. More accurate classifications could need to depend more strongly on anatomical, biomolecular and biogeographical data.

Bivalve systematics and studies of bivalve evolution have been based historically upon conchological characters because mollusc collections, both recent and fossil, consist primarily of dry shells. Shells are durable, and relatively easy to transport, preserve and examine. Thus, bivalve evolution has often been discussed in terms of conchological diversity adapting to environmental diversity (e.g. Stanley, 1970, 1977a, 1981).

But how often do conchological similarities reflect genetic versus adaptive similarities? How large a role does adaptive parallelism play in evolution? Such questions have led to the development of evolutionary systematics, a school that argues that systematics should be based on genetic, not morphological similarities. Thus, systematics is intended to reflect the evolutionary processes of species and higher taxa, and not merely the groupings of morphologically similar objects (De Queiroz, 1988; Lindberg, 1989).

Biomolecular techniques have provided some insight into adaptive parallelism, and resulting phylogenies of various extant groups of animals (e.g. Sibley and Ahlquist, 1991; Lowenstein, 1985; Jope, 1980) suggest that such parallelism is more prevalent than previously suspected. Parallelism was an important component in the early evolution of Bivalvia (Stanley, 1974); apparent cases occur frequently throughout Cardiidae (Savazzi, 1985) and Veneridae. More knowledge about the nature of the process might be gained by specifically examining how and where parallel evolution actually occurs throughout the evolution of a large successful family of organisms.

There are inherent limits in examining adaptive

parallelism. Both "parallel" and "convergent" have been used to describe the acquiring of superficially similar characters through evolutionary time as similar adaptations to similar selective pressures by genetically distant taxa. When interpreting data on clusters of extant taxa, such a phenomenon can only be inferred, because data on ancestral species are absent. Depending on the definition of the points of reference, the inference can be wrong. The superficially similar but genetically distant taxa of A and B, for example, could appear to be convergent. Yet the immediate ancestor of A could be much more similar to that of B, so that A and B are really diverging currently, even if their overall history is one of convergence. Thus, to advance beyond inference, both extinct and extant points of reference must be defined, as well as the scale and scope of characters that comprise the presumed parallelism. For most studies, such data are rarely available. To succeed, fossils of ancestral taxa are needed from which both morphological and genetic data can be extracted.

Opportunities to do this within the Bivalvia exist through the biomolecular technique radio-immuno-assay (RIA). RIA measures immunologically genetic distance between taxa by measuring how much antibodies made against proteins of one species bind with proteins from another. The method is comparable roughly to DNA hybridization in that the products derived from the DNA, the proteins, are essentially hybridized via the mediation of the immunological reaction. The immunological distances resulting from RIA correlate highly with DNA hybridization (Sibley and Ahlquist, 1991), microcomplement fixation (Lowenstein *et al.*, 1981)

and DNA sequencing results (Lowenstein and Scheuenstuhl, 1991). RIA can detect extremely small amounts of protein (Luft and Yalow, 1974) and can distinguish closely related species (Lowenstein *et al.*, 1981; Lowenstein and Ryder, 1985) or compare widely divergent groups (Lowenstein, 1981). A unique feature of RIA is that it can provide information on proteins preserved in fossils as old as 60 million years (Westbroek *et al.*, 1979) and in calcareous matrices (Lowenstein, 1981; Lowenstein *et al.*, 1982, 1991; Molleson, 1982; Rainey *et al.*, 1984; Collins *et al.*, 1988). RIA has been used successfully on such disparate groups as algae (Olsen-Stojkovich *et al.*, 1986), gymnosperms (Price *et al.*, 1987), brachiopods (J. M. Lowenstein, pers. comm.), and reptiles, birds, and mammals (Lowenstein, 1981).

Within the Bivalvia, the Veneridae (Heterodonta: Veneracea) is an example of a large, global, diverse family with a rich fossil record; 500 or more extant species are classified into approximately 12 subfamilies, with 50 extant and 55 extinct genera, and 150 extant and 99 extinct subgenera. The earliest venerid fossils are approximately 130 million years old. Present in a wide variety of marine ecosystems, members of the family are characterized by having three cardinal teeth in each valve, and sometimes up to three anterior teeth (one in the left valve and two smaller interlocking ones in the right). A lunule, escutcheon, and pallial sinus are usually present; valves have concentric sculpture ranging from smooth to pronounced, and sometimes radial and divaricate sculpture, as well.

Venerid taxonomy is controversial, with several discrepancies among recent systematic works (Fischer-Piette and Delmas, 1967; Keen, 1969; Fischer-Piette, 1975; Fischer-Piette and Vukadinovic, 1975, 1977). No consistent, comprehensive conchological descriptions exist for the subfamilies, genera and subgenera. Genera, especially of minute clams, continue to be moved among subfamilies or changed by workers (Bernard, 1982; Lindberg, 1989), who must weigh which characters indicate true phylogenetic alliance versus parallel adaptations.

Little genetic information exists on the Veneridae. RNA sequencing data exist on three taxa (Bowman, 1989) but the amount is inadequate to draw significant conclusions about the family. Phylogenetic estimations are mostly based on conchological characters (e.g. Parker, 1949; Casey, 1952; Fischer-Piette and Vukadinovic, 1977) or shell microstructure (Shimamoto, 1986). The taxonomic confusion, lack of genetic information, and the size, age and diversity of Veneridae all highlight the potential gains to be had through RIA in understanding the evolution and systematics of the family.

Geographic and anatomical data indicate parallel evolution within Veneridae. Jones (1979) anatomically examined four species of the sub-family Chioninae: the west Pacific *Chione (Austrovenus) stutchburii* (Wood), the west Atlantic

Chione cancellata (Linnaeus) and *Mercenaria mercenaria* (Linnaeus), and the east Pacific *Chione californiensis* (Broderip). The genus *Chione* is characterized by large, well-spaced concentric cords or lamellae, and strong radial ribs on the valves. *Mercenaria* has fine, closely spaced concentric threads that merge medially into smooth areas but can develop into weak, low lamellae laterally on the valves; a prominent, rugosely sculpted nymph is also present (Figs. 1f-g). Jones (1979) observed that anatomically, the three North American species (the western Atlantic Ocean and eastern Pacific Ocean) were allied much more closely to each other than to *C. stutchburii*, despite the conchological differences, indicating either a parallelism in anatomy among the American chionines, or in conchology between *C. stutchburii* and the American *Chione* spp.

Similarly, Keen (1969) places two geographically disparate but conchologically similar species, *Anomalocardia brasiliana* (Linnaeus) and *Cryptonomella producta* (Anton) within the chionine genus *Anomalocardia* Schumacher, which she characterized as thick shells, with undulating concentric folds crossed by radial riblets, and large, impressed lunules. Harte (1992) observed similarities in sculpture, profile and nymphs between *Anomalocardia s. s.* and species of *Chione* and *Mercenaria*, however, and *A. brasiliana* overlaps geographically with *Chione cancellata*, which might indicate a common genetic origin. Past workers have occasionally classified *Anomalocardia* as a subgenus of *Chione* (Olsson, 1932; Parker, 1949).

As a preliminary test of RIA on venerid shells, I used RIA to determine whether the above two cases inferred conchological parallelism. The results are presented here.

MATERIALS AND METHODS

RIA data were obtained for two groups of taxa (Fig. 1) to test for parallel evolution. Nomenclature follows that of Keen (1969). The first group included: *Chione cancellata* (CC), *C. (Austrovenus) stutchburii* (CS) and *Mercenaria mercenaria* (Linnaeus) (MM). Another west Pacific chionine species, *Timoclea (Glycydonta) marica* Linnaeus (TM) was also included. The second test group included *Anomalocardia brasiliana* (AB) and *A. (Cryptonomella) producta* (PR), which were compared with the first group. *Macrocallista nimbosa* (Lightfoot) (NI) (Veneridae: Pitarinae), was used as an outgroup to the two test groups of chionines.

RIA analyses on the above species were carried out under the direction of Dr. Jerold Lowenstein at the Medical Center of the University of California at San Francisco, where he has refined the method with over a decade of research on various groups of organisms. For each species, approximately 10 g of shell was ground to a powder and placed in 100 cc of 0.2M EDTA, a calcium chelating agent, for two days to dissolve the CaCO₃. The resulting protein solution was then

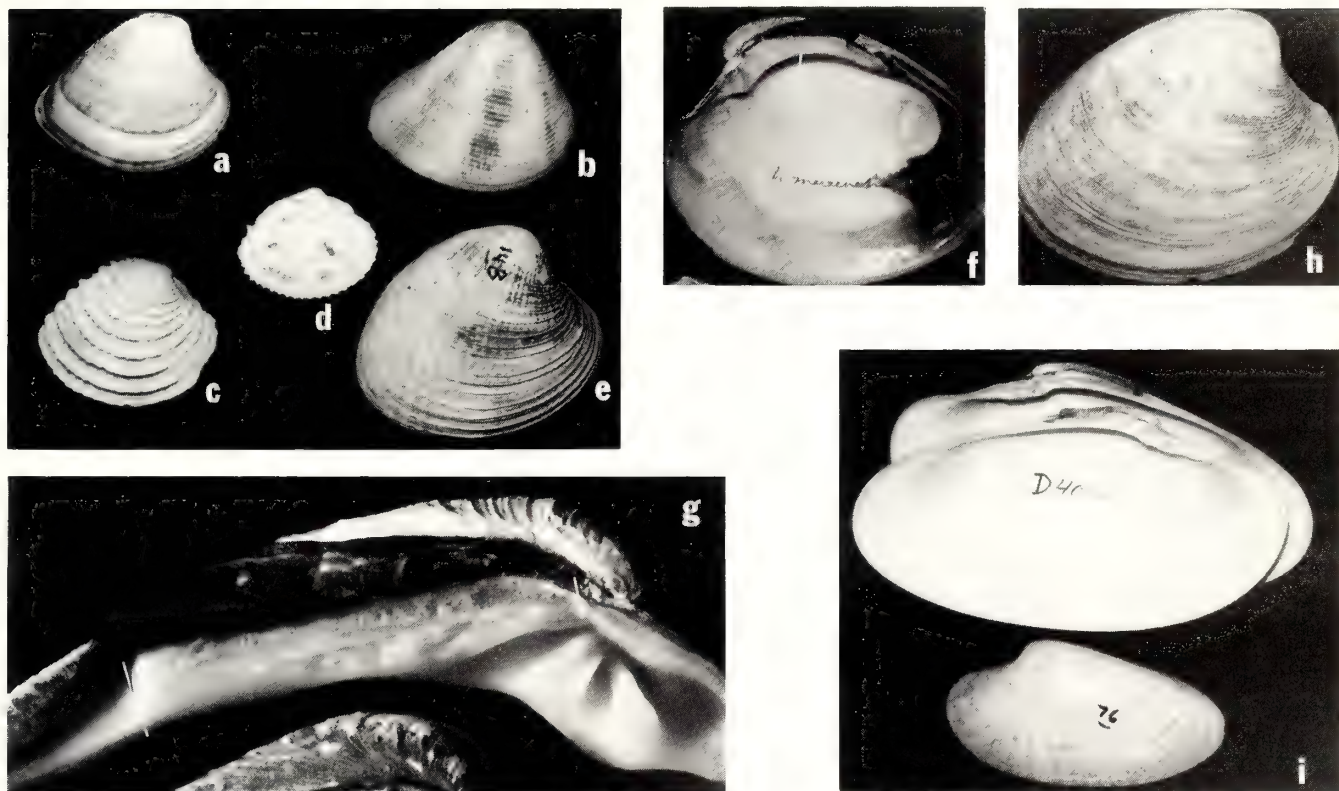


Fig. 1. a. *Anomalocardia brasiliana*, [shell length (s.l.) = 3.0 cm]; b. *A. (Cryptonemella) producta* (s.l. = 3.2 cm); c. *Chione cancellata* (s.l. = 2.8 cm); d. *Timoclea marica* (s.l. = 2.2. cm); e. *C. (Austrovenus) stutchburii* (s.l. = 3.8 cm); f-h. *Mercenaria mercenaria*. f. interior with rugose nymph indicated by white bar; g. rugose nymph (section between white bars = 2.2 cm); h. exterior (s.l. = 6.8 cm); i. *Macrocallista nimbosa* (s.l. = 8.6 cm, top; 5.5 cm, bottom).

tested for adequate reactivity against a pre-existing antibody made against *Arctica islandica* Linne. If the test was successful, a rabbit was injected at two points along its neck with 2.0 cc total of equal parts shell protein solution (antigens), and Freund's adjuvant, an agent used to stimulate antibody response. Every two weeks thereafter, the rabbit was further injected with 1.0 cc of shell protein solution. At the end of two months, the rabbit was drained of its serum, containing the antibodies made against the shell proteins.

A 0.1 dilution of the antigens for each species was made and tested against the antibodies of all the species. Antigens of species A were placed in a cup of a plastic microtiter plate and allowed to bind with the plastic for one hour. The excess was removed, and the cup was coated with 0.5% soy serum for five minutes to bind with the remaining plastic not bound by antigens A'. Excess serum was removed, and the rabbit antibodies of species B were added to the cup to bind with antigens A' for two days. Excess antibodies were removed, and the cup was rinsed with soy serum again. Radioactively tagged Iodine-125 goat antibodies produced against rabbit gamma globulin (GARGG) were then added to the cup for one day to bind with the antibody B-antigen A' complexes

present. The cup was then rinsed with water, dried, placed inside a radiation counter tube, and its radioactivity measured. Additionally, for each set of antibodies, control cups without antigens were treated as above to determine the levels on nonspecific binding of the antibodies to the soy rinse serum. The radioactive count of an empty radiation counter tube represented the radioactive standard, the level of background radioactivity.

The resulting matrix of raw data was adjusted by first subtracting the nonspecific binding levels of the controls from tests involving their respective antibodies. The matrix was then divided by the radioactive standard, and the resulting quotients were expressed as percentages. The immuno-distance (ID) for each reaction was calculated as follows:

$$ID_{A'B} = 100 \log_{10} (A'A/A'B);$$

$$ID_{AB'} = 100 \log_{10} (B'B/B'A);$$

$$ID_{AB} = 1/2 (ID_{A'B} + ID_{AB'});$$

where A'A, for example, is the reaction of the antigens (A) with the antibodies (A') of species A. A Fitch-Margoliash unrooted tree of the taxa was calculated from the resulting lower diagonal ID matrix using the program PHYLIP 3.1.

RESULTS

Results of the reactivity tests indicated that protein content varied noticeably among individual shells from the same species and even from the same lot, with some shells often not having adequate amounts of protein. Unknown and differing concentrations of antigens and antibodies do not interfere with interpretation of the resulting immunological distances, however, because of the nature of the immunological reaction, the experimental design, and the immunological distance algorithm. In RIA, the immunological reaction is a two step process: a plastic substrate is coated with antigen, which is subsequently exposed to reaction by antibodies. Because the binding substrate for antigens has a high affinity and limited capacity for antigens, the first step is a saturation reaction that requires little dissolved protein (i.e. antigen) to saturate all sites. Thus, different protein concentrations still result in saturation of binding sites. (This explains the fact that in preliminary trials, some antigen solutions ultimately yielded functioning antibodies, despite previous reactivity tests indicating that these solutions had practically no proteins present). The binding of the antibodies to antigens is an equilibrium reaction, however, so the amount of binding is proportional to antibody concentration. For different antibody concentrations, this is compensated by the counter reactions, which are incorporated as proportions into the immunological distance algorithm. Consider the example of antigens of species A reacting against two concentrations of antibodies from species B: weak ($B'B = 20$, $B'A = 4$), and strong ($B'B = 40$, $B'A = 8$). The resulting ID_{AB} remains unchanged because, as explained above, the concentrations of antigens A and B do not affect their reactions with the antibody, A'. The resulting $ID_{AB'}$ also remains unchanged, whether the strong or weak antibodies are used, because the $B'B/B'A$ term is equivalent in both cases ($20/4 = 40/8 = 5$). Thus, ID_{AB} remains unchanged, regardless of the concentration of antibody used.

While shells recently separated from their animals (i.e., within days) exhibited adequate amounts of protein, there were no other clearcut predictors of adequate protein levels. Beachworn specimens often did not have adequate amounts of protein. Adequate protein solutions of all the above species were obtained, however, and antibodies were made against them.

Results are presented in Table 1 and figure 2. The matrix indicates that a deep division exists between Chioninae and Pitarinae, represented by NI, although NI is allied with the chionine taxa through TM, with the distance between NI and TM, 42, comparable to other distances among the chionine taxa (e.g. distances between TM and MM, and CS and MM). The unrooted Fitch-Margoliash tree groups the six chionine taxa in three pairs: AB and TM, CC and MM, PR and CS. All three pairs are conchologically dissimilar; the second pair are west Atlantic species and the third pair

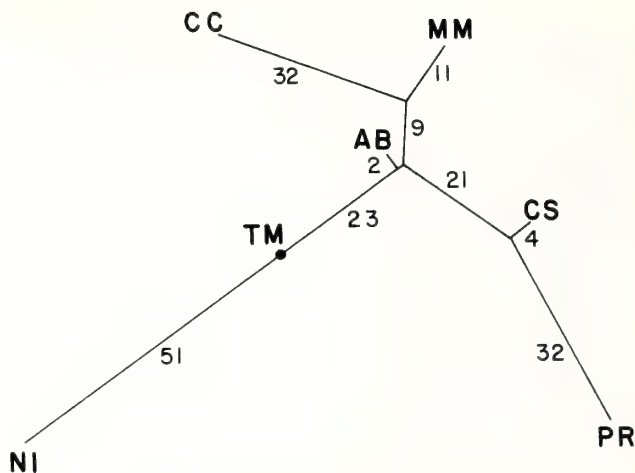


Fig. 2. An unrooted Fitch-Margoliash tree derived from Immunological Distance data on venerid taxa (abbreviations in text) (S. D. = 17.3, S. S. = 1.2).

are west Pacific species. Using CC as a point of reference, the tree indicates parallel evolution of either the cancellate sculpture of Chione twice in CS and TM or parallel evolution of the rostrate posterior and primarily concentric sculpture of *Anomalocardia* once in PR. Because no data are available on ancestral species, neither possibility assumes priority or an arbitrary direction. The matrix indicates that AB and TM are most closely allied (25), followed by PR and CS (39), and CM and MM (43). The matrix data agree well with the anatomical analyses of Jones (1979).

DISCUSSION

Much has been observed about bivalve adaptations (e.g. Carter, 1968; Stanley, 1970; Seilacher, 1974; Savazzi, 1985). The burrowing paradigm of Seilacher (1974) required that valve sculpture be perpendicular to the direction of burrowing, asymmetrical in crosssection, and reduced medially (perimeter smoothing). Later experimentation and observations have supported this paradigm (Stanley, 1977b; Stanley, 1981; Savazzi, 1985). A clam burrows anteriorly, and assumes a life position with the posterior closest to the sediment surface. From this it is logical to assume that the anterior will facilitate burrowing and anchorage. The posterior, especially of shallow burrowers, coming into contact with the substratum only towards the end of burrowing, can contribute little towards it, and in cardiids posterior sculpture often does not conform to the burrowing paradigm (Savazzi, 1985). Being the point closest to the surface and predators, however, the posterior probably functions more towards predatory defense and reducing surface scour of sediment around the shell, thereby preventing disinterment. These are useful perspectives for analyzing venerid adaptations.

Most chionine clams burrow sluggishly and shallow-

Table 1. Immunological distances among various venerid taxa.

		<i>Chione stutchburii</i>	<i>Timoclea marica</i>	<i>Mercenaria mercenaria</i>	<i>Chione cancellata</i>	<i>Anomalocardia brasiliiana</i>	<i>Anomalocardia producta</i>	<i>Macrocallista nimbosa</i>
Group 1	<i>Chione stutchburii</i>							
	<i>Timoclea marica</i>	55						
	<i>Mercenaria mercenaria</i>	46	43					
	<i>Chione cancellata</i>	59	106	34				
Group 2	<i>Anomalocardia brasiliiana</i>	28	25	27	39			
	<i>A. producta</i>	37	77	77	103	56		
Outgroups	<i>Macrocallista nimbosa</i>	128	42	134	163	87	112	

ly, with the posterior tip positioned within 1 cm of the sediment surface (Stanley, 1970). The shells are moderately thick, prosogyrous, and subovate with a slightly angular posterior; most have strong valve ornamentation. In each species, the unique set of variations among these characters reflects a unique balance and compromise of adaptive strategies. This is illustrated in the above results, which not only indicate conchological parallelisms among west Pacific and North American chionine clams, but an extensive conchological diversification between closely related species.

The latter is especially well illustrated between *Mercenaria mercenaria* and *Chione cancellata*. *M. mercenaria* is a large, thick shelled, moderately rapid burrower (Stanley, 1970) of subdued, predominantly concentric sculpture. Such sculpture aids burrowing, while size and thickness help keep it anchored in the sediment (Kauffman, 1969). The clam adjusts burrowing depth (1-2 cm between posterior and sediment surface) and life position to sediment type, and inhabits an unusually broad range of environmental conditions (Stanley, 1970); this ability to adapt to sediment changes probably accounts at least partly for its wide exploitation of habitats. In contrast, *C. cancellata* is a small, thick shelled, slow burrower (Stanley, 1970) with well spaced, sharp, concentric lamellae, slightly corrugated from underlying, well spaced radial ribs. Its life position is with the posterior near or at the surface, and its habitat is comparatively restricted (Stanley, 1970). Stanley (1981) showed that the strong sculpture inhibited burrowing. Furthermore, a rough comparison of the ornamentation and burrowing rate indices of these and other Caribbean *Chione* species (Stanley, 1970) indicates that burrowing rate is inversely correlated to the development of the concentric sculpture, a relationship supported on a broader scale throughout bivalvia (Kauffman, 1969). The strong sculpture of *C. cancellata* compensates posteriorly by reducing scour, however (Stanley, 1981); once interred, the sharp lamellae probably aid anchorage and discourage nipping of the short siphons or firm grippage by predators. Radial ribs not only confer rigidity to the shell (Kauffman, 1969) but in this case evidently strengthen the lamellae basally and the resulting corrugations of the lamellae (Stanley, 1981).

Lack of life history data precludes comprehensive comparisons of conchological features and life strategies for the pair of west Pacific chionines, *Chione stutchburii* and *Anomalocardia producta*. While they have different profiles and concentric sculpture, they have similar radial patterns. Radial ribs are virtually absent anteriorly, appearing faintly medially and predominating the posterior sculpture. Posterior radial ribbing offers an acceptable approximation to being perpendicularly oriented to water currents, thereby reducing surface scour (Savazzi, 1985), which might explain its presence in both species, although not as a parallel adaptation but as a commonly derived one. The concentric sculpture in both species is more subdued than that of *C. cancellata*, indicating that it functions in them more as a burrowing aid than anchor. For the more streamlined species, *C. stutchburii*, it could enable more rapid burrowing, enabling exploitation of less stable habitats; indeed, the species inhabits a wide variety of sediments, ranging from less stable sediments of sand and gravel to a more stable muddy sand within estuaries and enclosed bays (Beu and Maxwell, 1990). For *A. producta*, concentric sculpture might compensate at least partly for its lack of streamlining.

The third linked pair of chionines, *Timoclea marica* and *Anomalocardia brasiliiana*, are the most closely linked of the pairs immunologically, yet exhibit wide disparities in profile, sculpture and geography. Their different adaptive pathways exhibit interesting parallels. Both live near the surface, are similar in size and probably equally slow infaunal burrowers. *T. marica* has sculpture closely similar to *Chione cancellata*: well developed lamellae (though not as widely spaced) that flare posteriorly, corrugated by well separated radial ribs. A plausible inference is that it functions similarly, inhibiting burrowing but serving as an anchor, an anti-scour mechanism posteriorly, and to discourage predators.

Similar functions in *Anomalocardia brasiliiana* probably are accomplished through its unusual profile rather than its relatively subdued sculpture, which aids burrowing. *A. brasiliiana* and *Chione cancellata* are equally slower burrowers (Stanley, 1970); the blunt, relatively obese anterior profile probably inhibits burrowing. The compensations, however, are several. Angular to rostrate posteriors serve to

elevate the siphonal flow with a minimum of shell excretion while permitting the center of gravity to remain relatively deep, although streamlining is sacrificed (Stanley, 1970). The rostrate posterior maximizes this effect. The life position in the sediment is perpendicular to the sediment surface (Stanley, 1970), and this minimizes the posterior surface vulnerable to scouring and predators. Such advantages could result in parallel selection for this trait, and, indeed, this trait appears in several subfamilies of Veneridae [e.g. *Lepidocardia floridella* (Gray), Pitarinae; *Eumarcia paupercula* (Holten), Tapetinae; *Timoclea malonei* (Vanatta) and *T. peresi* Fischer-Piette and Vukadinovic, Chioninae].

Another difference between *Timoclea marica* and *Anomalocardia producta* is that the posterior dorsal margins of *T. marica* are finely crenulated, while those of *A. producta* slightly interlock by means of a long, shallow fold in the right valve. Hypotheses on defensive functions of crenulated margins include increasing resistance of the shell to compression from shell-crushing predators (Waller, 1969), restricting predatory access of starfish, and creating a tight seal (Carter, 1968), thereby preventing release of diagnostic chemicals into the environment, and increasing survival times in a predator's digestive tract (Vermeij, 1987). Restricting predatory access and creating a tight seal might be effected equally by marginal folding, and function similarly. Jones (1979) observed, for example, that marginal folding effectively keeps the posterior dorsal margin closed while siphons are extended, and suggests that the resulting marginal overlaps might deter polychaete pests. Additionally, both marginal interdigitation and folding can thicken the marginal juncture, discouraging boring predators. Which adaptation is ultimately chosen might depend ultimately on slight differences in ontogeny and environment.

The data indicate, then, that *Timoclea marica* and *Anomalocardia brasiliensis* are closely related species exhibiting different sculpture, profiles and posterior margins that function similarly in aiding anchorage and discouraging predators. In both species, efficiency in burrowing is sacrificed for advantages in anchorage and predatory discouragement.

The Fitch-Margoliash tree indicated parallel evolution of either rostrate posteriors and their accompanying characteristics, or cancellate sculpture. Arguments for the former are advanced, above. Parallel adaptation of the cancellate sculpture characteristic of *Chione* and *Timoclea* could occur in various species because of the cumulative advantages offered by ribbing, and the ontogenetic ease with which strong, well spaced concentric sculpture can be modified into structures that aid anchorage and defense (lamellae) or burrowing (cords or ridges), facilitating evolution into different life strategies. Indeed, this transition can be seen within several species of venerids, such as *Mercenaria mercenaria*, where juveniles, more vulnerable to disinter-

ment, have widely spaced low, anchoring, concentric lamellae (Pratt and Campbell, 1956) that gradually become closely spaced, more subdued threads, medially worn smooth in adults.

The RIA data have several ramifications for venerid systematics, which, for the above chionines, is beset with definitional problems. As developed by Keen (1969), the genus *Anomalocardia* is paradoxical: except for *Anomalocardia s. s.*, the concentric sculpture of the included subgenera are concentric cords, not the undulating folds given in the generic definition (Fig. 1a-b). The result is a de facto assemblage of chionine species with rostrate posteriors and primarily concentric sculpture. Immunological distances indicate the features are a parallel adaptation, although the tree allows an interpretation of them as a derived adaptation.

Besides having different concentric sculpture, *Anomalocardia producta* differs from *A. brasiliensis* in lacking a rugose nymph and crenulated margins, and in having pronounced posterior radial ribs. While not as obvious a trait as a rostrate posterior, the rugose nymph of *A. brasiliensis* is a distinct marker for linking the taxon conchologically to immunological allies, such as *Mercenaria*.

Matrix data support the morphological transition proposed by Harte (1992) linking *Anomalocardia s. s.* to *Mercenaria* and two subgenera of *Chione*, *Lirophora* and *Ilioichione*, and *Mercenaria*. Besides a rugose nymph, present at various degrees in all four taxa, specific traits include concentric undulations as the predominant sculpture, with a posterior that ranges from angular (*Mercenaria*) to rostrate (*Ilioichione*, some *Lirophora* and *Anomalocardia*).

Several systematic changes are indicated by the above data. Matrix data indicate that *Cryptonomella* is more closely related to *Chione* (*Austrovenus*) *stutchburii* than *Anomalocardia*; it should stand as a separate genus until it can be demonstrated to be closely linked to a senior genus. *C. (A.) stutchburii* is more closely allied to *Anomalocardia* and *Mercenaria* than to *Chione* and should stand as a separate genus until its intergeneric relationships are further clarified. The relationships of the other subgenera of *Chione* (*Chionista* Keen, 1958; *Chionopsis* Olsson, 1932; *Panchione* Olsson, 1964 and *Securella* Parker, 1949) should be clarified before deciding the systematic relationships of *Chione* to other chionine taxa.

The immunological distances between *Macrocallista nimbosa* and most of the chionine taxa indicate a deep division within the family, an observation of systematic workers, as well. With some exceptions, venerid taxa fall roughly into two groups: 1) those with well developed anterior lateral teeth, and simple valve sculpture (Pitarinae, *et al.*); 2) those with little or no anterior lateral teeth, and often strong valve sculpture (Chioninae, *et al.*). Although venerid classification has been in a state of constant flux, some past workers have tended to sometimes merge existing subfamilies within these

two groups (Fischer, 1887; Dall, 1902; Jukes-Browne, 1914). In contrast, Frizzell (1936) elevated the ten subfamilies that existed by then into separate families. Keen (1969) returned them to subfamilies, and the relatively close alliance of *M. nimbose* to *Timoclea* indicates that the family is monophyletic.

CONCLUSIONS

Radio-immuno-assay has been used successfully in conjunction with conchological and anatomical analyses to help illustrate various evolutionary processes within Veneridae. RIA and conchological analyses of Pacific and Atlantic Ocean chionines indicate that between distantly related species there exists one or more strong conchological parallelisms, which have as their bases adaptations towards predatory defense, positional stability, and burrowing efficiency.

RIA analyses also indicate that closely related species have undergone extensive conchological diversification. This diversification probably results from adaptation to different lifestyles, or utilizing different adaptations for similar functions. The conchological differences between *Mercenaria mercenaria* and *Chione cancellata* reflect different life strategies for predatory defense and positional stability. *M. mercenaria* appears to rely on burrowing abilities, size and thickness, while *C. cancellata* relies on sculpture, thickness, and a more specialized habitat. Conchological differences between *Timoclea marica* and *Anomalocardia brasiliensis*, in contrast, reflect different conchological strategies that effect parallel adaptive compromises, and cope with parallel lifestyles.

Ramifications for systematics include separating *Cryptonomella* from *Anomalocardia*, separating *Austrovenus* from *Chione*, and reassessing the systematic relationships of *Anomalocardia*, *Timoclea*, *Mercenaria* and *Chione*. A deep division exists between the subfamilies Pitarinae and Chioninae, although a single, relatively close link between them indicates that the family is monophyletic. Geographic and anatomical data, and obscure conchological traits can sometimes indicate genetic alliances, and should be utilized in assessing systematic relationships.

RIA has the potential for proving examples of parallel evolution between extant bivalve taxa and their ancestors. Further research utilizing fossil taxa is planned.

ACKNOWLEDGMENTS

I gratefully acknowledge the technical assistance of Dr. Jerold Lowenstein and Gary Scheuenstuhl. Materials for this study were donated by the Museum of Paleontology, University of California at Berkeley, and the Museum of Comparative Zoology, Cambridge. I thank Dr. John Harte, Dr. David Lindberg, and Dr. Jerold Lowenstein and two anonymous reviewers for their constructive advice and criticism. This research was supported under NSF grant BSR-9009189, through the Rocky Mountain Biological Laboratory.

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Date of manuscript acceptance: 25 November 1991

Research on marine bivalves in the People's Republic of China

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Abstract. This paper summarizes the published literature on marine bivalves in the People's Republic of China from 1950 to 1991. The 221 citations listed in the bibliography contain only significant works as viewed by the author. The summary includes systematic research on 23 families of bivalves distributed in the Chinese seas and encompasses the Arcidae, Glycymerididae, Limopsidae, Mytilidae, Pinnidae, Pteriidae, Isognomonidae, Pectinidae, Plicatulidae, Ostreidae, Chamidae, Cardiidae, Tridacnidae, Mactridae, Mesodesmatidae, Tellinidae, Solenidae, Veneridae, Myidae, Corbulidae, Pholadidae, Teredinidae and Laternulidae.

The breeding biology and experimental ecology of 12 economically important bivalves is addressed for the following species; *Crassostrea rivularis* (Gould), *Crassostrea gigas* (Thunberg), *Mytilus edulis* (Linnaeus), *Perna viridis* (Linnaeus), *Sinonacula constricta* (Lamarck), *Tegillarca granosa* (Linnaeus), *Chlamys farreri* (Jones and Preston), *Chlamys nobilis* (Reeve), *Argopecten irradians* (Lamarck), *Ruditapes philippinarum* (Adams and Reeve), *Pinctada martensii* (Dunker), and *Pinctada maxima* (Jameson).

China is a large coastal country, with 18,000 kilometers of coastline, a vast expanse of seas, and over 14,000 kilometers of islandic coastline. There are four seas, the Bohai Sea, the Yellow Sea, the East China Sea and the South China Sea, with an area of 4.75 million square kilometers, including more than 6,000 islands. Marine bivalve resources are very rich throughout the region.

Three marine molluscan faunal provinces for China seas have been identified: 1) a warm temperature region that includes the Yellow Sea and Bohai Sea; 2) a subtropical region that includes the East China Sea, the north-western coast of Taiwan and the northern coast of Hainan; 3) a tropical region including the south-eastern coast of Taiwan, the coast of the southern tip of Hainan Island and the area south of them.

The suffix to this paper offers more than 200 citations concerning Chinese marine bivalves. This obviously does not include the entire Chinese bivalve literature but rather a circumspect summary of the significant works. The majority of the papers were written in Chinese, with English abstracts or with English headings only, though a small number were written in English. There have also been several articles that were published in general magazines, but are not included in this summary.

SUMMARY OF MARINE BIVALVE RESEARCH IN CHINA

1. Systematics and Biogeography

In China, the study of marine bivalve taxonomy started much later than in western Europe. Before October 1949 (The Liberation), foreign and domestic scientists had published

few comprehensive systematic works. However, between 1950-1990, 40 years were dedicated to intensive sampling and investigation along China's coastal seas, that is, starting from the most northern sea, the Yalu River Estuary, to the southern most area, the Xisha Islands. Between 1958-1960, a nationwide general exploration was completed in the coastal seas; from 1959 to 1962 an investigation on the Beibu Gulf of the South China Sea was completed. Owing to the extensive number of sampling stations and frequency of seasonal investigation, a large number of specimens were obtained. These specimens have been identified and are housed currently in the Institute of Oceanology, Academia Sinica. Thanks to the extended collecting plan, we have been able to report on the distribution of Chinese coastal and near sea bivalves and summarize the zoogeography of the region. From 1980-1984, under the leadership of Chinese National Bureau of Oceanography, these investigations were completed. The above work was done by relevant universities of the coastal provinces, each with a mission to collect and preserve large numbers of samples. The investigation of the coastal regions was also combined with sublittoral zone research (0 to 5 m). Further, since 1990, the Chinese National Bureau of Oceanography has been organizing the coastal provinces to launch large-scale islandic investigations. Further study is anticipated on the coastal resources, followed by a program of marine bivalve resource development and utilization.

Marine bivalves hold an important position among marine molluscs, due to their use as a human food and as feed for domestic fowl and prawns, and thus they are viewed as a key resource in the national economy. Our research is based primarily on bivalve species that can be developed for

a market economy or have some economic impact, such as members of the Ostreidae and Teredinidae. Many articles have been published in China about the breeding, growth and ecology of *Crassostrea rivularis* (Gould, 1861), and *Teredo navalis* Linnaeus, 1758, resulting in enhanced oyster breeding and a shipworm prevention and treatment program.

Additional research on marine fisheries, physical oceanography, and pollution has yielded large numbers of specimens that can be used for detailed studies of the systematics, ecology and zoogeography of bivalves. On the basis of these scientific materials, taxonomists have published many monographs and treatises on a variety of families and genera, and have discovered and described many new genera and new species. As basic survey work continues, taxonomists continue to engage in systematic research on the families that have economic value, such as the Arcidae, Mytilidae, Pinnidae, Pectinidae, Mactridae, Veneridae, Tellinidae, Pholadidae, Corbulidae, Laternulidae and Myidae.

2. Biology and Ecology

The biology and ecology of intertidal and infaunal benthic bivalves has been superficially studied. Naturally, there has been some analysis of the variation in mollusc species composition and density. The ecology and biology of local species of *Donax*, *Moerella* and *Cultellus* has been studied, and the population structure of *Perna viridis* (Linnaeus, 1758) has been examined in several regions.

General research on benthic community structure in three inner bays in the Bohai Sea, has yielded data on the relatively high densities of *Scapharca subcrenata* (Lischke, 1869); *Chlamys farreri* (Jones and Preston, 1904), *Atrina pectinata* (Linnaeus, 1758), and *Paphia undulata* (Born, 1778), suggesting possible sites to develop and utilize in the future.

3. Experimental ecology and mariculture of economically important species

Oysters. The literature on Ostreidae breeding far exceeds that of other families. The primary species utilized in China are *Crassostrea rivularis* and *C. gigas* (Thunberg, 1793). *C. rivularis* is common in the Pearl River Estuary, and is the primary species harvested in Guangdong Province. It is also found in lower densities in the Fujian Province. Results of research on the feeding habits, breeding seasons, predators, and artificial rearing of larvae are included in the literature reviewed below. Beside the traditional cultivation method of "throwing stones", a new raft culture technique has produced high yields. *C. gigas* is a well known species in the Fujian Province, and reports on its culture were recorded in Xiapu County in the early 16th Century. The annual harvest of this species in 1986 was 44,200 tons.

Mussels. There are three species of economically important mussels in China: the Purple mussel, *Mytilus edulis* Linnaeus,

1758; the Hardshell mussel, *M. crassitesta* Lischke, 1868; and the Jade mussel, *Perna viridis*. *M. edulis* is distributed in north China, *M. crassitesta* is distributed in Liaoning, Shangdong and Zhejiang Provinces, but *P. viridis* is found only in Fujian and Guangdong Provinces.

Cultivation of marine mussels began in China in 1958, at which time studies were undertaken to study mytilid life history, feeding habits, sexual maturation, growth, meat condition, method of culture, development of larvae, and artificial rearing of spat. Mussel rearing conditions, feeding requirements and treatment of larval culture water has been reported in numerous publications. Artificial spat rearing of these three mussel species has been successful.

The primary mussel breeding technique in China utilizes hanging rafts. Due to the large acreage devoted to culture, juveniles are usually collected from sea-weed rafts. New types of ecologically sound breeding programs have been tested, such as combined mussel-seaweed culture, where the nitrogenous waste products of mussels are utilized as fertilizer for the sea-weed, and in turn the growth of the seaweed improves the mussel culture.

Chinese Razor Clam. *Sinonovacula constricta* (Lamarck, 1818) lives in coastal waters with lower salinity than usually found in the northern and southern mid-littoral zones, however, artificial breeding of razor clams has been successful in the Zhejiang and Fujian Provinces. Traditional culture techniques have been practiced in those areas where the juvenile clams naturally settle, allowing easy collection, redistribution and harvest. By the end of the 1950's, malacologists had examined the reproductive biology of razor clams. Analysis of these data has provided an effective forecast system for rake flat productivity, and has allowed the establishment of harvest quotas. In this way, the number of clams harvested has been increasing. From 1970-1980, malacologists experimented with natural juvenile collection. These studies included monitoring temperature, salinity, the relationship between rate of survival of juvenile clams and growth, as well as the influence of the environment on the rate of razor clam incubation and the rate of gonad maturation.

Ark Clams. *Tegillarca granosa* (Linnaeus, 1758) is widely distributed along the Chinese coast, with artificial breeding mainly occurring in the Zhejiang, Fujian and Guangdong Provinces. Traditionally, the Shangdong Province supplies juvenile bloodclams that are then dispersed in the mid-lower intertidal zone and fed until they reach a preset size, after which they are spread, taking 1-2 years to reach marketable body weight (500 gms/80 shells). A production of 750 - 4,500 kg/mu is an optimistic yield. Extensive study on this species has been neglected and further investigations are desirable.

Scallops. Three species within the Pectinidae are commercially

exploited in China, *Chlamys farreri* (Jones and Preston, 1904), *C. nobilis* (Reeve, 1852) and *Argopecten irradians* (Lamarck, 1819). *C. farreri* is distributed throughout the Yellow Sea and Bohai Sea, as well as to the south of the East China Sea, along the eastern Zhejiang coastal sea. *C. nobilis* is mainly found in the coastal area of the Fujian and Guangdong Provinces, and *A. irradians* has been introduced from the United States.

The adductor muscle of *Chlamys farreri* is the only tissue used in the production of dried scallop muscle, *ganbei*. In the mid-1950's, the famous Chinese malacologist, Tchang Si, developed methods for studying the feeding and growth of scallops, and provided a good foundation for the culture of this species. In 1974, Chinese scientists began to collect natural scallop spat and successfully experimented with artificial feeding and cultural production of *C. farreri*. In 1979, artificial culture experiments produced 0.92 million spat/m². Many research laboratories in North China have continued investigations of reproductive cycles, natural spat collection, artificial breeding and the study of an immense standard seeding, which has pushed the scallop fishery to the verge of mass production. During this period, breeding methods were changed from rafting and hanging techniques to field spreading.

Chlamys nobilis is an economically important species that has been cultivated in South China. Owing to the success of artificial breeding, cultivation has been modified to a method similar to the one used in mussel culture, that is, growing scallops on seaweed rafts.

Argopecten irradians is an important species that originated from the Atlantic Ocean. This species grows rapidly and produces a marketable scallop (mean length of 5 cm) in one year. In 1982, Professor Zhang Fusui (Institute of Oceanology, Academia Sinica) introduced breeding stock of *A. irradians* and has had excellent results in North China, with a higher fecundity than usual. The species has gradually spread down to northeast Fujian, creating a system of autumn artificial spat setting suitable to the thermal limitations in South China. Currently scientists are conducting a series of large scale experiments on the development of high density spat-rearing for this species. In 1984, Professor Zhang and his colleagues introduced the Japanese scallop, *Patinopecten yessoensis* (Jay, 1857) into China in hopes of utilizing this species for the production of dried scallop adductor muscles (*ganbei*).

Littleneck Clam or Flower Clam. *Ruditapes philippinarum* (Adams and Reeve, 1850) is distributed broadly along the Chinese coast. In the north, the intertidal and sub-tidal zones have large beds of this clam, however, this is primarily due to the presence of strict resource protection and planned gathering. Artificial culture has been seldom practiced. Nevertheless, the Fujian Province has an important project

on the artificial breeding of the Littleneck clams, including research on reproduction mechanisms, spermatogenesis, influences of chemicals and sexual products on hastening parturition, life history, growth of spats, and feeding habits. Laboratory breeding success, combined with mass production needs, have facilitated experiments in outside earth ponds including egg production, larval cultivation, and mass field production.

Pearl Oysters. The major species in China is *Pinctada martensii* (Dunker, 1850), which is used in pearl culture and is an important enterprise in Guangdong, Guangxi and Hainan Provinces. Research on this species has encompassed artificial breeding techniques including nucleus insertion, induced polyploidization, and observation of gonads during triploidization. Scientists have successfully completed inter-species hybridization of *P. martensii*, *P. chemnitzii* (Philippi, 1847) and *P. maxima* (Jameson, 1901), and have studied hybrid chromosomes and their zymograms. Many studies have been undertaken on *P. maxima*. Owing to its scarce distribution in nature, large shell, and good quality and high priced pearls, artificial breeding experiments have been completed. However, it must be noted that many hurdles still must be overcome to utilize this species.

Along with the seven important bivalves mentioned above, research has begun on the breeding and ecology of other bivalves such as *Coelomacra antiquata* (Spengler, 1802), *Meretrix meretrix* (Linnaeus, 1758), *Macra veneriformis* Reeve, 1854, *Musculus senhousei* (Benson, 1842), *Atrina pectinata* (Linnaeus, 1758), *Laternula marilina* (Reeve, 1860), *Saxidomus purpuratus* (Sowerby, 1852), *Scapharca broughtoni* (Schrenck, 1867) and *Potamocorbula laevis* (Hinds, 1843).

CONCLUSIONS

The study of systematics has naturally focused on families and genera that are economically important, leaving ignored several important families without commercial potential. Limited by restricted resources, we have only described species along the Chinese coast. Most studies have been limited to the identification of species, morphological descriptions, geographical distributions and a discussion of nomenclatural problems. The systematic treatment of superfamily and subspecific levels and the evolutionary relationships of bivalves have been inadequately studied. Moreover, most systematic research has been based chiefly on external morphology. The study of shell microstructure and functional morphology has been meager.

Due to the over emphasis of research on commercial species and the limited funding in general, the study of bivalve ecology has been neglected, especially research on autecology and synecology. Much attention has been paid to bivalve

experimental ecology and breeding, but spat ecology needs further study. Facing the outside world and absorbing Euro-American advanced technology is required to enhance Chinese cultivation of marine bivalves.

ACKNOWLEDGMENTS

I thank Ms. Lin Huiqiong who helped collect the literature and critically read the manuscript. Paul Scott of the Santa Barbara Museum of Natural History and Robert Prezant of Indiana University of Pennsylvania provided assistance with editing and translating this article. Eugene Coan of the Los Angeles County Museum consulted on the cited literature.

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- Date of manuscript acceptance: 24 April 1992

AMERICAN MALACOLOGICAL UNION FINANCIAL REPORT

1991 Income and Expenses

Income:

1990 Dues (all categories)	\$ 3,626.00
1991 Dues (all categories)	11,931.00
1992 Dues (all categories)	9,167.00
Life Membership	400.00
<i>Bulletin</i> Subscriptions - Volume 8	1,817.00
<i>Bulletin</i> Subscriptions - Volume 9	2,375.00
<i>Bulletin</i> Subscriptions - Volume 10	1,364.00
<i>Bulletin</i> Subscriptions - Volume 11	32.00
<i>Bulletin</i> back issues & Supplements	839.00
Page charges and reprints	1,930.85
Other publications	193.00
T-shirt sales	1,557.00
Logo items	188.00
Student Award Donation	250.00
Auction	806.41
Interest (all accounts)	3,492.57
Miscellaneous income	466.88
Symposium Fund Donations	1,150.00
	<u>\$41,585.71</u>

Expenses:

Office expenses	1,937.63
Postage	1,437.75
Dues in other organizations	345.00
Franchise and Corporation Fees	100.00
Insurance and Bond	383.00
Accounting	280.00
Travel Expenses	2,065.00
I.R.S.	140.00
Bank charges	159.28
Symposium expenses	2,968.00
T-shirts	932.90
Student Paper Awards	750.00
1991 Meeting expenses	331.20
Logo items	118.45
<i>Bulletin</i> 8(2)	6,273.87
<i>Bulletin</i> - reprints for 8(1), 1990	140.45
Newsletters (two)	2,130.03
	<u>\$20,492.56</u>

Increase in cash assets	21,093.15
Cash assets as of 1/1/91	76,948.81
+ 1991 increase	21,093.15
	<u>\$98,041.96</u>

IN MEMORIAM

Anita Brown Croft
Ralph W. Dexter
MacKenzie Gordon, Jr.

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Dates of Publication

Volume 9(1), December, 1991

Volume 9(2), August, 1992

CONTRIBUTOR INFORMATION

The *American Malacological Bulletin* serves as an outlet for reporting notable contributions in malacological research. Manuscripts concerning any aspect of original, unpublished research, important short reports, and detailed reviews dealing with molluscs will be considered for publication.

Each original manuscript and accompanying illustrations must be submitted with two additional copies for review purposes. Text must be typed on one side of 8½ x 11 inch bond paper, double-spaced, and all pages numbered consecutively with numbers appearing in the upper right hand corner of each page. Leave ample margins on all sides.

Form of the manuscript should follow that outlined in the *Council of Biology Editors Style Manual* (fifth edition, 1983). This can be purchased from the CBE, 9650 Rockville Pike, Bethesda, Maryland 20814, U.S.A.

Text, when appropriate, should be arranged in sections as follows:

1. Cover page with title, author(s) and address(es), and suggested running title of no more than 50 characters and spaces. Authors should also supply five key words, placed at the base of this page, for indexing purposes.
2. Abstract (less than 5% of manuscript length)
3. Text of manuscript starting with a brief introduction followed by methodology, results, and discussion. Separate sections of text with centered subtitles in capital letters.
4. Acknowledgments
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All binomials must include the author attributed to that taxon the first time the name appears in the manuscript [e.g. *Crassostrea virginica* (Gmelin)]. This includes non-molluscan taxa. The full generic name along with specific epithet should be written out the first time that taxon is referred to in each paragraph. The generic name can be abbreviated in the remainder of the paragraph as follows: *C. virginica*.

References should be cited within text as follows: Hillis (1989) or (Hillis, 1989). Dual authorship should be cited as follows: Yonge and Thompson (1976) or (Yonge and Thompson, 1976). Multiple authors of a single article should be cited as follows: Beattie *et al.* (1980) or (Beattie *et al.*, 1980).

In the literature cited section of the manuscript references must also be typed double spaced. All authors must be fully identified, listed alphabetically and journal titles must be unabbreviated. Citations should appear as follows:

- Beattie, J.H., K.K. Chew, and W.K. Hershberger. 1980. Differential survival of selected strains of Pacific oysters (*Crassostrea gigas*) during summer mortality. *Proceedings of the National Shellfisheries Association* 70(2):184-189.
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R.A. Lutz, eds. pp. 23-67. Plenum Press, New York.

Yonge, C.M. and T.E. Thompson. 1976. *Living Marine Molluscs*. William Collins Son & Co., Ltd., London. 288 pp.

Illustrations should be clearly detailed and readily reproducible. All line drawings should be in black, high quality ink. Photographs must be on glossy, high contrast paper. All diagrams must be numbered in the lower right hand corners and adequately labeled with sufficiently large labels to prevent obscurity with reduction by one half. Magnification bars must appear on the figure, or the caption must read Horizontal field width = xmm or xµm. All measurements must be in metric units. All illustrations submitted for publication must be fully cropped, mounted on a firm white backing ready for reproduction, and have author's name, paper title, and figure number on the back. All figures in plates must be nearly contiguous. Additional figures submitted for review purposes must be of high quality reproduction. Xerographic reproduction of photomicrographs or detailed photographs will not be acceptable for review. Abbreviations used in figures should occur in the figure caption. Indicate in text margins the appropriate location in which figures should appear. Color illustrations can be included at extra cost to the author. Original illustrations will be returned to author if requested.

Any manuscript not conforming to *AMB* format will be returned to the author for revision.

New Taxa. The *Bulletin* welcomes complete descriptions of new molluscan taxa. Establishment of new taxa must conform with the International Code of Zoological Nomenclature (1985). Descriptions of new species-level taxa must include the following information in the order as given: higher taxon designation as needed for clarity; family name with author and date; generic name with author and date; *Genus species* author sp. nov. followed by numeration of all figures and tables; complete synonymy (if any); listing of type material with holotype and any other type material clearly designated along with complete museum catalogue or accession information; listing of all additional non-type material also with full museum deposition information; type locality; diagnosis and full description of material done in telegraphic style including measurements and zoogeographic distribution as necessary; discussion; etymology. Descriptions of new supra-specific taxa should include type species (for new genus) or type genus (for new family), diagnosis and full description done in telegraphic style, and list of included taxa.

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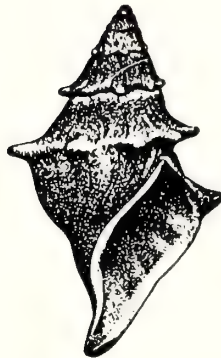
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